Fuel Selection in Genetically Selected Endurance Running Rats at Submaximal Exercise Intensities

by

Kristina Murphy

A Thesis Submitted to the School of Graduate Studies In Partial Fulfillment of the Requirements For the Degree
Master of Science

McMaster University

April 2007

Master of Science (2007) McMaster University
Department of Biology

Title: Fuel Selection in Genetically Selected Endurance Running Rats at Submaximal Exercise Intensities

Author: Kristina Murphy

Supervisor: Dr. Grant McClelland

Number of Pages:

ABSTRACT

Exercise intensity is one of the major factors determining the utilization of carbohydrates (CHO) and lipids in mammalian skeletal muscle. Using indirect calorimetry, we determined maximal oxygen uptake (VO_2max) and whole-body rates of CHO and lipid oxidation in rats selectively bred for high and low running capacity (HCR's and LCR's) during exercise at 50, 60, 70 and 80%VO_2max. Previous studies have revealed a pattern of selection where mammals with different aerobic capacities use the same proportions of lipids and CHO when exercising at the same relative exercise intensity and as intensity increases, CHO use increases and lipid use decreases. The present results showed that the HCR's had a VO_2max and distance run to exhaustion that was 1.3 and 4.0 times greater than the LCR's respectively. Also, both groups of rats followed the pattern of fuel selection seen in previous studies where the same proportions (in %) of lipids and CHO are used at the same relative exercise intensity. On an absolute scale, the HCR's used more lipids and CHO than the LCR's at all exercise intensities but the results were not always statistically significant. We also determined the exercise intensity that elicited the greatest lipid use to be 60%VO_2max in both groups.

In order to explain these patterns of fuel selection, metabolic indicators, metabolites and enzymes, in skeletal muscle were measured at rest and post exercise for one hour at 60%VO_2max. Specifically, ATP and phosphocreatine (PCr) metabolite concentrations were determined in the medial and lateral gastrocnemius, extensor digitorum longus (EDL), tibialis anterior (TA), and soleus muscle. The medial gastrocnemius and soleus were analyzed (pre and post exercise samples were combined) for their oxidative and glycolytic enzyme activity by measuring citrate synthase (CS),

cytochrome oxidase (COX), β-hydroxyacyl CoA dehydrogenase (HOAD), and lactate dehydrogenase (LDH) . PCr and ATP concentrations did not change pre and post exercise and between the HCR's and LCR's except for the EDL where there was a significant decrease ($P<0.05$) in both metabolites after exercise in both groups of rats. For the enzyme measurements, CS and COX activities were higher ($P<0.05$) in the HCR's for the soleus and HOAD activities were also higher in the HCR's medial gastrocnemius compared to the LCR's. We concluded that the HCR's have a greater oxidative capacity as shown by their greater aerobic and endurance capacity (VO_2max and distance to exhaustion), their ability to oxidize a greater absolute amount of lipids and CHO's at the same relative exercise intensity, and their higher activities of oxidative enzymes in the soleus (CS and COX) and medial gastrocnemius (HOAD). Future research into the mechanisms involved in explaining these patterns of fuel selection may include examining fatty acid transport proteins, fatty acid and CHO availability, fiber types, and catecholamines.

Acknowledgements

This project enabled me to develop and strengthen skills that are not only applicable to biology but also to life. I gained a greater understanding of the field of physiology and in the process, learned more about myself, especially my strengths and weaknesses.

I would like to give thanks to my supervisor, Dr. Grant McClelland, my committee members, Dr. Rollo and Dr. Gibala, and my labmates for all of their guidance during my time here.

TABLE OF CONTENTS

List of Figures

CHAPTER 1: INTRODUCTION

Carbohydrates (CHO) and lipids are the primary substrates involved in producing ATP to power muscle contraction during exercise in mammals. Proteins are not thought to contribute significantly to overall energy production during locomotion (Rennie *et al.*, 2006). Exercise intensity is one of the major determinants of fuel selection; in mammals, a pattern of fuel selection occurs where CHO and lipids are used in the same proportions during the same relative exercise intensities (percentages of maximal oxygen uptake), even in animals of differing aerobic capacities. The purpose of this study was to determine how exercise intensity affects fuel selection in rats selectively bred for their intrinsic endurance running capacity (high and low) and in turn, determine if there are any metabolic changes in skeletal muscle. The introduction consists of an overview of energy production, fuel selection, and the regulation of CHO and lipids during exercise, along with the metabolic adaptations associated with increased endurance capacity, and an introduction to the unique rat model used in this study.

1.1 ENERGY (ATP) PRODUCTION DURING EXERCISE

In order to maintain metabolic homeostasis during submaximal exercise, ATP utilization must be matched by ATP production and this is accomplished by the oxidation of CHO's and fats. During exercise above VO_2max (sprinting), ATP use and production are not matched and ATP concentration falls. When exercise begins the phosphagen system provides a limited supply of ATP for the first initial seconds of exercise through the reaction: $PCr + ADP + H^+ \leftrightarrow Cr + ATP$ catalyzed by Creatinephosphokinase (CPK). A larger supply of ATP needed for prolonged exercise is then provided by the oxidation

of CHO's and fats through glycolysis, β-oxidation, Kreb's cycle, and the electron transport chain (ETC). At low to moderate exercise intensities up to approximately 65%VO_2max, ATP is ultimately provided aerobically by oxidative phosphorylation in the ETC. In this process, pyruvate produced by glycolysis is converted to acetyl-CoA by pyruvate dehydrogenase (PDH) and is fed into the Krebs cycle. Acetyl-CoA from glycolysis and β-oxidation of lipids enters the Kreb's cycle and reducing equivalents $NADH^+$, H^+, and $FADH_2$ carry electrons to be passed through the ETC for energy production. Since the demand for ATP is not that high at low to moderate intensity exercise, the system is able to keep up with ATP production (Spriet *et al.*, 2000).

At moderate to high intensity exercise, there is a mismatch in energy utilization and production. The greater demand for ATP at moderate to high intensities results in increased glycolytic flux leading to the accumulation of pyruvate (because it is not being oxidized fast enough through PDH), and resulting in lactate formation by the reaction: pyruvate + NADH ↔ lactate and NAD catalyzed by lactate dehydrogenase (LDH) (Spriet *et al.,* 2000). ATP production occurs in this manner until fatigue occurs.

1.2 FUEL SELECTION DURING EXERCISE

1.2.1 Exercise intensity and fuel selection

Exercise intensity and duration not only have an affect on which metabolic pathways are used to produce energy but also which fuels are oxidized to generate ATP. Exercise intensity is an expression of how hard the body is working while doing exercise and can be described as being absolute or relative (Holloszy *et al.* 1998). Relative exercise intensities are expressed as percentages of maximal aerobic capacity or

VO_2max; two different individuals with different aerobic capacities who are exercising at the same relative intensity are producing energy at different rates. Absolute work rates determine the total amount of fuel required to power muscle contraction during exercise (Holloszy *et al.* 1998). For example, altitude acclimated rats with a lower VO_2max have a lower rate of lipid oxidation than sea level rats running at the same speed. Sea level rats, because of their higher aerobic capacity, are exercising at a lower relative intensity than the altitude rats at the same speed, therefore oxidizing a greater amount of lipids (McClelland *et al.*,1999). In this example, absolute work rates are determined by speed. In the present study, relative exercise intensities (percentages of maximal O_2 uptake) will be used to determine fuel selection so each rat will be running at a different speed.

Exercise intensity as a determinant of fuel selection was noticed as early as 1934 when Dill and colleagues observed a greater reliance of CHO accompanied by a decreased reliance on lipids, to power muscle contractions at higher exercise intensities (Edwards *et al.*, 1934). Since then, the crossover concept has been constructed to develop this idea further in humans (Brooks and Mercier, 1994). The crossover concept explains that at low to moderate exercise intensities, lipids are the main fuel powering exercise and at higher exercise intensities (>65%VO_2max), CHO become the dominant source of fuel, even in endurance trained athletes (Brooks and Mercier, 1994). Indeed, studies in humans have shown that at lower exercise intensities (~40%max), fatty acid oxidation dominates (Wolfe *et al.*, 1990) and reaches a maximum at 65%VO_2max (Achten *et al.*, 2002; Romijn *et al.*, 1993) and high exercise intensities (~80%VO_2max) are fueled primarily by CHO's in humans and rats (Romijn *et al.*, 1993; McClelland *et*

al., 1998) resulting in a drastic decline in fat oxidation above 89%VO_2max (Achten *et al.*, 2002).

1.2.2 Fuel selection patterns in mammals

Patterns of fuel selection based on relative exercise intensity are seen in a variety of animals tested so far. Specifically, there is growing evidence that the same proportions of carbohydrates (CHO) and lipids are used by animals of different aerobic capacities when they are exercised at the same percentage of their VO_2max (McClelland, 2004). For instance, dogs have greater than twice the aerobic capacity of goats. Theoretically endurance capacity could be enhanced by increasing lipid use since these fuels can be stored in large amounts and are more reduced than CHO. It is therefore reasonable to assume that dogs would use different proportions of lipids than goats to power exercise. This prediction however is not true; in exercise studies comparing these two species, proportional use (%VO_2) of fuel are found to be the same and this pattern is consistent across exercise intensities (McClelland *et al.*, 1994; Weber *et al.*, 1996).

Similarly, to determine the effect of environmental O_2 availability on fuel use, McClelland *et al.* (1998) determined the contribution of CHO during 1 hour of exercise at 60 and 80%VO_2max in rats at sea level (SL) and rats acclimated to high altitude (HA). Interestingly, percent contribution of CHO oxidation to overall energy expenditure was the same between the two groups (McClelland *et al.*, 1998). Similarly, in a subsequent study (McClelland *et al.*, 1999) high altitude acclimated SL and HA rats were found to oxidize the same percentage of lipids at 60 and 80% of their VO_2max during 25 to 60 minutes of treadmill running (McClelland *et al.*, 1999).

Human exercise studies also support the notion of conserved fuel selection. For example, Bergman *et al.,* (1999) tested glucose kinetics during 1 hour of cycling at 45 and 65% VO_2max in untrained and trained men who underwent 9 weeks of cycle ergometer training. Despite differences in training status, it was found that whole body glucose disposal and uptake rates did not differ between trained and untrained men at 65%VO_2max. On an absolute basis, glucose disposal and uptake rates were attenuated post training (Bergman *et al.*, 1999).

From these examples, data across taxa, body size and varying aerobic capacity show strikingly similar patterns of fuel selection relative to exercise intensity (see McClelland, 2004). Generally, the mammals tested so far all derive the same fraction of their total energy from carbohydrates and lipids when exercising at the same % VO_2max.

1.2.3 Exercise duration and fuel selection

In conjunction with exercise intensity, the duration of exercise has an effect on which fuels are used to power exercise. During prolonged exercise, there is a gradual shift from CHO to fatty acid metabolism. In humans, fatty acids become the dominant source of fuel contributing 62% of the total fuel after 40 minutes of exercise at an intensity of 30%VO_2max (Ahlborg, 1974). At higher exercise intensities, ranging from 55 to 75%VO_2max, fatty acids become dominant after approximately 90 minutes, taking over from intramuscular lipids and glycogen after 30 minutes when these fuels begin to deplete (Holloszy *et al.* 1998). Rats running for 1 hr at 60-63%VO_2max and swimming until exhaustion at 48%VO_2max have an increased concentration of plasma FFA's and glycerol, and a decreased glycogen content in the liver and muscle (Koubi *et al.* 1991).

Clearly, as exercise continues, there is a greater reliance on fatty acids for energy in both humans and rats.

1.2.4 Protein contribution during exercise.

In comparison to CHO and lipids, protein is not a significant contributor of energy during exercise. In humans, studies suggest that protein contributes about 3% to total energy expenditure in endurance-trained athletes (Phillips *et al.*,1993; Gibala, 2001). More specifically, endurance training was shown to cause a decrease in leucine oxidation and branched-chain 2-oxoacid dehydrogenase activity (enzyme that oxidizes leucine) (McKenzie *et al.*, 2000). In rats, it has been shown that protein contributes about 4.2% to exercise in the untrained state and increases in the trained state to 6% (Henderson *et al.*, 1985). Although protein is not an important contributor to energy supply, studies have shown that amino acids are involved in reactions with Kreb cycle intermediates to contribute to aerobic energy production at the beginning of exercise (for review see Gibala, 2001). For the purposes of this study, the rats being studied will not be in a trained state and it is assumed that protein will not be a significant contributor of energy during exercise.

1.3 REGULATION OF CHO'S AND LIPIDS DURING EXERCISE

1.3.1 Lipid metabolism during exercise

As mentioned, lipids are an important source of energy during low exercise intensities and during prolonged exercise. The regulation of lipid metabolism is still under debate because many of the mechanisms are still unclear. Lipids are predominately

stored in adipose tissue but they are also found in the plasma as lipoproteins, and in the muscle as intramuscular triglycerides (IMTG's). Fatty acids (FA's) derived from adipose tissue will be discussed in greater detail than IMTG's and lipoproteins because they are the most relevant to this study.

When exercise begins, epinephrine and norepinephine stimulate lypolysis (breakdown of triglycerides) by activating hormone sensitive lipase (HSL). Non-esterized fatty acids (NEFA's) and glycerol are released from the triglycerides and out of the adipocyte and enter into circulation. Some of the FA's are re-esterified and are stored as triglycerides in adipose tissue while other FA's are bound to albumin in the plasma and transported to the muscle cell to be oxidized. Currently, the transport of FA's from the circulation to the cell cytosol is unclear. Long chain fatty acids and their entry into the cytosol may be accomplished by a transporter protein, passive diffusion, or both (Kiens, 2006). The fatty acid proteins that are implicated to participate in this process are the fatty acid binding protein plasma membrane (FABPpm), fatty acid translocase (FAT/CD36), or fatty acid transport protein (FATP) (Kiens, 2006). FAT/CD36 will be discussed in more detail in the discussion. Much of the evidence suggests that at the plasma membrane, FAT/CD36 aids in the transport of FA's into the cell and then they are bound to FABPpm (Luiken *et al.*, 2002). FA's are then transformed to fatty acyl-CoA's by the enzyme acyl-CoA synthase and transported to the mitochondrial membrance attached to an acyl-CoA binding protein. Fatty acyl-CoA is then transported across the outer mitochondrial membrane by carnitine; carnitine palmitoyltransferase I (CPT-I) catalyzes the conversion of fatty acyl-CoA to fatty acyl-carnitine Fatty acyl-carnitine is then transported into the mitochondria matrix by carnitine-acyl transferase and then CPT-

II (on the inner mitochondrial membrane) catalyzes the conversion back to fatty acyl-CoA to be oxidized by β-oxidation (McClelland, 2004). Short and medium chained FA (C2 to C12) do not require the carnitine system for transport across the inner mitochondrial membrane and instead diffuse into the matrix and are converted to their Co-A derivatives and subsequently oxidized (Nelson, 2000). There are many potential sites of regulation in the pathway leading to FA oxidation and it seems that FA uptake is the main regulatory step in this process. Entry into the mitochondria catalyzed by CPT-I is considered a controlling step and also is any step involving transporters (McClelland, 2004).

The use of IMTG's during exercise is a controversial issue mainly because of the discrepancies in the techniques used to measure IMTG's (Watt *et al.*, 2003) and the various factors involved that affect their use during exercise including exercise intensity and duration, age, gender, and training status. Despite this, studies in endurance-trained males have revealed that they are an important source of energy during exercise (Krssak *et al.*, 2000; van Loon, 2003). Krssak *et al.*, (2000) showed that trained individuals who underwent 45 minutes of running on a treadmill at 65-70%VO_2max had significantly decreased IMTG content after 2-3 hours of exercise. Similarly, endurance-trained athletes cycling at 60%VO_2max had a 21 % decrease in IMTG content post exercise after a low fat and normal diet. IMTG's seem to be an important source of energy during exercise, at least in endurance trained humans.

In other mammals, endurance training increases IMTG use in dogs and goats when exercising at 40 and 60%of their VO_2max for one hour (Weber *et al.*, 1996). Also, the more aerobic dog (greater VO_2max) relies even more on intramuscular fuels than the

goat when exercising at the same relative intensity (Weber *et al.*, 1996). IMTG use during exercise is another area of fat metabolism that requires further research.

1.3.2 CHO metabolism during exercise

The CHO used during exercise are found in the blood as glucose and in the muscle and liver as glycogen. Blood glucose is an important energy source during low exercise intensities and during longer exercise periods when glycogen stores are depleted. Glucose supply to the muscle tissue and transport into the muscle cell are key regulatory steps in the overall metabolism of CHO (Rose and Richter, 2005). Exercise causes a large increase in blood flow which is important for the perfusion of glucose into muscle tissue however the concentration of plasma glucose seems to be the limiting factor for the uptake of glucose during exercise (Rose and Richter, 2005). Glucose transport is accomplished by the protein GLUT-4 and is stimulated via muscle contraction and insulin stimulation. Although it is known that an increased amount of GLUT-4 transporters increases glucose transport in the muscle cell, the regulation of how this GLUT-4 upregulation occurs by muscle contraction and insulin is still uncertain (Rose and Richter, 2005).

Muscle glycogen is the primary substrate for exercise at high intensities. Strenuous exercise is accompanied by an increase in the rate of glycogenolysis (glycogen breakdown) due to calcium-mediated glycogen phosphorylase activation. An increased concentration of Ca^{2+} converts glycogen phosphorylase to its active form via phosphorylase kinase. Phosphorylase kinase becomes active by the actions of protein kinase A which itself is stimulated by an increase in cyclic AMP due to increased

amounts of catecholamines (Holloszy *et al.* 1998). When glycogen is broken down, it too is used to produce ATP via glycolysis, Kreb's cycle, and the ETC.

1.3.3 Interaction of Lipid and CHO metabolism during exercise

The interaction between lipid and CHO metabolism during exercise is currently not understood. Most of the evidence points to CHO and lipid availability in controlling which fuels are used during exercise (Spriet and Howlett, 1999). One of the early mechanisms that was proposed is the glucose-fatty acid cycle or Randle cycle which focuses on the influence of increased fat oxidation on CHO metabolism (Randle *et al.*, 1963). Randle *et al.* (1963) used isolated rat heart to show that an increase in fat oxidation increased the acetyl-CoA/CoA ratio thereby inhibiting pyruvate dehydrogenase (PDH) activity and subsequently reducing the amount of acetyl-CoA available for the Kreb cycle. Increased fat oxidation also lead to an increase in citrate concentration which inhibits phosphofructokinase (PFK), and decreases flux through glycolysis by allowing glucose-6-phosphate to accumulate and inhibit hexokinase (HK). This ultimately prevents the entry of glucose into the cell to be oxidized for energy (Randle et al., 1963).

Currently, other studies using FA concentration manipulations have been used to investigate the interaction between fat and CHO metabolism. For example, Odland *et al.*, (1998) increased FFA availability, through intralipid infusions, during low (40%VO_2max) and moderate (65%VO_2max) intensity exercise in eight males in various states of training, to determine if CHO sparing occurred and the mechanisms involved. The main finding was that increasing FA's during moderate intensity exercise reduced glycogenolysis by 23%. Particularly, citrate concentration increased , glycogen

phosphorylase activity decreased, AMP and ADP contents were reduced, and there was a decreased transformation of PDH to PDHa. Glucose uptake was unaffected and there was no increase in muscle acetyl-CoA. These results suggest that a high FA availability may be affecting CHO metabolism at glycogen phosphorylase possibly due to decreased ADP and AMP contents, PDH, and possibly PFK due to an increased citrate concentration (Odland *et al.*, 1998).

Stellingwerff *et al.* (2002) decreased FA availability by using a FA inhibitor to look at PDHa activation in eight active males undergoing 40 minutes of exercise at 55%VO_2max. The results revealed that a decreased availability of FA's during exercise resulted in an increased respiratory exchange ratio (RER), CHO oxidation, and PDHa activation. According to the authors, the increased PDHa activity was not due to changes in pyruvate or ATP/ADP ratio but may have occurred because of a reduced $NADH/NAD^+$ ratio or increase in Ca^{2+} possibly due to epinephrine (Stellingwerff *et al.* 2002).

Increased glucose concentrations have also been used to investigate the regulation of the interaction between CHO and lipid metabolism. Coyle, 1997 used hyperinsulinemic subjects (dietary manipulation) to demonstrate that CHO availability regulates fat oxidation during exercise at 50%VO_2max for 40 minutes. The results showed that CHO availability during exercise inhibited LCFA oxidation and had no effect on medium-chained fatty acid oxidation (Coyle, 1997). Possible mechanisms involved in the understanding of how an increase in glucose concentration regulates lipid metabolism has been studied in rat skeletal muscle. For instance, an increase in glucose concentration has been shown to increase citrate concentration (rapidly) and in turn,

increase malonyl CoA (M-CoA) (Saha *et al.*, 1997; Kraegen *et al.*, 2006). Also, glucose can activate acetyl-CoA Carboxylase (ACC) by a decreasing AMP-activated protein kinase (AMPK) activity which occurs more slowly (Kraegen *et al.*, 2006). ACC has been shown to regulate the concentration of M-CoA because it catalyzes the formation of M-CoA from cytosolic acetyl-CoA (ACC_{β} in skeletal muscle). M-CoA is a potent inhibitor of carnitine palmitoyltransferase I (CPT-I) which, as mentioned above, is the enzyme that regulates the transport of long-chain fatty acyl-CoA into the mitochondria (Saha *et al.*, 1997). If CPT-I is inhibited, fat oxidation has been shown to decrease.

Studies have used various manipulations (increase in fatty acid availability and glucose concentrations) to understand the interaction of glucose and lipid metabolism. Despite these insightful studies, the mechanisms involved are still not currently known.

1.4 METABOLIC ADAPTATIONS ASSOCIATED WITH AN INCREASED ENDURANCE CAPACITY

1.4.1 Endurance capacity and VO_2max

Endurance capacity is the ability to maintain steady-state exercise at a given work rate (Gonzalez, 2005) and is mainly determined by the muscle's oxidative capacity (increased mitochondrial content) (Bassett and Howley, 2000; Gonzalez, 2005). By increasing the activity of mitochondrial enzymes which are indicative of mitochondrial content, endurance performance is enhanced (Bassett and Howley, 2000).

VO_2max is the maximal rate of oxygen consumption or the highest oxygen uptake that an individual can obtain during exercise using large muscle groups (Powers and Howley, 1996). VO_2max is commonly used as an indicator of aerobic capacity

(Gonzalez *et al.*, 2005) and is determined primarily by the ability of the O_2 transport system to deliver O_2 to muscle mitochondria and secondly by the ability of the mitochondria to utilize oxygen (Bassett and Howley, 2000; Gonzalez, 2005). It has been shown to be a reliable measurement of aerobic capacity because it can be repeated in humans and in other mammals such as deer mice (Hayes and O'Connor, 1999).

Endurance capacity and maximum rate of oxygen consumption (VO_2max) do not necessarily correlate with one another (Bassett and Howley, 2000; Gonzalez, 2005; Coyle *et al.*,1988) because it is possible for two individuals to have the same VO_2max but have different amounts of mitochondria, enabling one person to have a greater endurance capacity than the other. In a study by Coyle *et al.*, (1988)endurance-trained competitive cyclists with similar VO_2max's had very different times to exhaustion, glycogen utilization, and blood lactate concentrations, all indicative of endurance performance. Instead, endurance performance was found to be related to the individual's VO_2 at their lactate threshold (Coyle *et al.*, 1988). Similarly, Gonzalez *et al.*, (2005) determined that the VO_2 max and distance run to exhaustion between the HCR's and LCR's did not correlate with one another and as a result, VO_2max and endurance capacity are controlled by different mechanisms (Gonzalez, 2005).

In the present study, VO_2max for the HCR's and LCR's was determined and used as a measurement of aerobic capacity while distance run to exhaustion is a measurement of endurance running capacity.

1.4.2 Regulation of gene expression to increase mitochondrial content

As mentioned above, an increase in muscle oxidative capacity is mainly due to an increase in mitochondrial content. This can be accomplished through changes in gene expression (as a result of training) associated with mitochondrial biogenesis. The exact mechanism of how this occurs is largely unknown however transcription factors such as the perioxisomal proliferator-activated receptor gamma coactivator-1α (PGC-1α) and the peroxisome proliferators-activated receptor (PPARγ) along with increases in cytosolic calcium have also been shown to stimulate mitochondrial biogenesis.

Evidence to support this comes from a study by Baar *et al.* (2002), who demonstrated that PGC-1 mRNA increased in rat skeletal muscle after endurance swimming training. Wang *et al.* (2004) targeted the expression of PPARγ and induced mitochondrial biogenesis, increased the number of type I fibers, and increased total RNA of oxidative enzymes, thereby creating a strain of mice capable of running long distances. Ojuka *et al.* (2002) subjected L6 myocytes to ionomycin ($Ca2^{+}$ ionophore), caffeine, and W7 (all which increase Ca^{2+}), intermittently for 5 hours per day. These exposures increased mitochondrial enzyme content and therefore mitochondrial biogenesis (Ojuka et al., 2002). Transcription factors and signals like calcium induce an adaptive response to a stimulus like training which causes the appropriate changes in gene expression (mitochondrial biogenesis) and in turn recruits certain levels of enzymes.

1.4.3 Enzymatic indicators of endurance capacity

Endurance capacity is determined by the muscle's oxidative capacity which can be determined by measuring mitochondrial content and oxidative enzyme levels. Many

studies use trained and untrained individuals to illustrate the effects of endurance training on muscle oxidative capacity. Endurance training typically results in significant improvements in VO_2max and increases in oxidative enzymes compared to untrained individuals (Bloomstrand, 1986; Henrikkson, 1977). Such enzymes include oxoglutarate dehydrogenase, succinate dehydrogenase (SDH), citrate synthase (CS), all found in the Kreb's cycle, and cytochrome oxidase (COX) in complex IV of the ETC, and 3-hydroxyacyl dehydrogenase (HOAD), an indicator of the capacity for fatty acid oxidation. CS, COX, and HOAD, increase their activity with endurance training in humans (Carter *et al.*, 2001); in mice, CS and COX activities also increase with endurance training (access to wheel running) (Houle-Leroy *et al.*, 2000). Endurance training clearly results in skeletal muscle adaptations at the enzyme level that increase endurance capacity.

1.4.4 Metabolite concentrations and endurance capacity

Metabolites are important signals in the cell that regulate metabolism by activating necessary pathways for energy production (Spriet and Howlett, 1999). During low exercise intensities, the demand for ATP is not that high and oxidative metabolism is able to keep up with energy production and therefore the rate of glycolysis is low. Pyruvate from glycolysis is mostly converted to acetyl-CoA instead of lactate and NADH and is used in the ETC. As a result, the concentrations of many metabolites compared to rest are only mildly affected; PCr use and lactate decrease, and free Pi, ADP, and AMP increase slightly (Spriet *et al.*, 2000).

During moderate to high intensity exercise, ATP utilization is not matched by production and as a result, there is an increase in the rate of glycolysis. PDH is unable to handle all of the pyruvate that is produced so some of it is converted to lactate along with NADH. This intensity also increases PCr use, and free ADP, AMP, and Pi accumulates. These metabolites activate glycolytic enzymes to support the increased flux to meet the energy demand (Spriet *et al.*, 2000).

Febbraio and Dancey (1999), measured various metabolites in six healthy, sedentary individuals who cycled to fatigue after approximately 3hrs at 64% VO_2max. Metabolites were measured at rest, 10 minutes, 40 minutes to fatigue, and at fatigue. It was found that lactate levels in both the muscle and plasma increased significantly at 10 minutes compared to rest but returned to resting levels 40 minutes until fatigue and at fatigue. Muscle glycogen was found to be significantly depleted at all time points and PCr decreased significantly at 40 minutes to fatigue and at fatigue compared to rest. ATP levels were maintained for the duration of exercise (Febbraio and Dancey 1999).

Endurance training has been shown to attenuate ATP and PCr use, lactate production, and the concentration of ADP, AMP, and Pi during exercise. For example, 7 weeks of training on a cycle ergometer for one hour at 75%VO_2max, resulted in decreased PCr use and lower levels of high energy phosphates like free ADP and AMP compared to pre-training (Leblanc *et al.*, 2004)..

Donovan and Brooks., (1983) and Donovan et al., (1990) showed that endurance training in rats resulted in greater lactate removal during easy and hard exercise or during lactate infusions of increasing concentration. In humans, endurance training reduced the appearance of lactate into the blood and increased its removal during low exercise

intensities but at higher intensities, there was only greater lactate clearance in comparison to untrained individuals (MacRae *et al.*, 1992). These studies support the notion that endurance training results is less disturbances in metabolism during exercise at the same intensity as pre-training.

1.4.5 Muscle fibers and oxidative capacity

Muscle fibers have different biochemical properties that confer their oxidative capacity and are activated depending on the energetic demands being placed on the muscle. In the rat, four fiber types have been identified; from the most oxidative to the least oxidative, type IIA>I>IID/X>IIB (Delp and Duan, 1996). The slow twitch oxidative fibers (IIA) contain many oxidative enzymes, mitochondria, capillaries, myoglobin, and have low ATPase activity. They are mainly recruited for aerobic metabolism. The fast twitch red fibers (IIA) have the greatest oxidative potential in the rat due to the strongest relationship seen between them and CS activity (Delp and Duan, 1996). The fast twitch glycolytic fibers (IIB and IID/X) contain a small number of mitochondria, have many glycolytic enzymes, have high myosin ATPase activity, and are less resistant to fatigue. These particular fibers have limited aerobic metabolism. The type IID/X fibers are intermediate between type IIA and type IIB in terms of oxidative potential (Delp and Duan, 1996; Armstrong, 1988; Armstrong and Laughlin, 1985). The soleus muscle contains predominately type I fibers and no type IIB or IID/X fibers. The largest percentage of type IIA fibers is located in the red gastrocnemius and the highest proportion of type IID/X fibers are in the extensor digitorum longus (EDL) (Delp and Duan, 1996).

In the rat, there is evidence to suggest that muscle fibers are distributed in a regular pattern and that they are recruited in that manner. In the rat, deep extensor muscles such as the soleus are mostly made up of slow-twitch fibers. Extensor muscles such as the gastrocnemius contain fast-twitch fibers on the superficial portion of the muscle and slow-twitch and fast-oxidative glycolytic fibers in the deep part of the muscle. With increasing movement in extensor muscles such as the progression from walking to running to sprinting, muscle fibers are recruited starting with the deep slow-twitch fibers to fast-oxidative glycolytic to fast-twitch fibers (Armstrong and Laughlin, 1985). The type of fibers and their recruitment pattern are important indicators of the oxidative capacity of muscles. This recruitment pattern also mirrors the pattern of fuel selection with progressively increasing exercise intensity in mammals (McClelland, 2004).

1.5 MODEL ORGANISM

1.5.1 High and low capacity rats (HCR's and LCR's)

A unique genetic model for intrinsic (untrained) aerobic capacity was developed from a genetically heterogenous founder population of 96 male and 96 female Sprague-Dawley rats (each from a different parentage) originating from a colony at the National Institute of Health (NIH:N, Koch and Britton, 2001).

In order to estimate aerobic running capacity, the rats underwent five trials of treadmill running until they reached exhaustion. Based on the best single trial of total distance run to exhaustion (in metres) on a treadmill, 13 low and 13 high capacity rats were selected and paired for mating. For the generations to follow, within-family

selection and rotational breeding was used to produce low and high lines (Koch and Britton, 2001).

With each new generation, it is evident that the HCR's responded more strongly to selection for endurance running capacity (Koch and Britton, 2001); the LCR's running capacity decreased 16m/min per generation and increased 41m/min in the HCR's (Wisloff *et al.*, 2005). Also, each generation revealed an increase in body weight in the low line and a decrease in body weight in the high line (Koch and Britton, 2001). The rat model currently employed for this present study represents the 16th generation of LCR's and HCR's. This model will provide insight into the genes associated with endurance running capacity and body weight, which are both important in health and disease.

1.5.2 LCR's as a model for disease

Many diseases are associated with imperfections in aerobic metabolism; aerobic capacity is therefore an important indicator of disease (Koch and Britton, 2005). Cardiovascular disease (CVD) is an example of such a disease and it encompasses a wide-range of disorders associated with the heart and blood vessels. Risk factors for CVD include metabolic abnormalities associated with obesity and diabetes, including elevated levels of fatty acids, hypertension, increased adipose tissue, and insulin resistance (Wisloff *et al.*, 2005).

With each new generation of selection for high and low running capacity, the LCR's gained weight and the HCR's lost weight (Koch and Britton, 2001). As a result, the LCR's are considered obese and are at a higher risk for CVD. The LCR's have a high mean blood pressure (13% greater than HCR's), high fasting insulin levels, and impaired

glucose tolerance which are associated with insulin resistance. They also have more abdominal adiposity, higher plasma triglycerides (VLDL's), and increased plasma free fatty acids (FFA) compared to the HCR's (Wisloff *et al.*, 2005). It has been shown that many of these metabolic abnormalities are associated with the metabolic syndrome, which includes obesity and diabetes (Sorrentino, 2005).

Wisloff *et al.*, (2005) measured 12 metabolic variables to determine aerobic running capacity and cardiac contractile function in untrained control and trained LCR and HCR rats and discovered that weight gain is responsible for abnormalities found in LCR rats. In the control groups, the LCR's had a 58% lower maximal aerobic capacity (VO_2max), 17% lower economy of running, and a 23% lower left ventricular weight compared to control HCR's. Also, mitochondrial impairment was evident in control LCR rats and this was seen through decreased amounts of transcription factors associated with the mitochondria including PPARγ and cytochrome c oxidase 1 (COX1). The control HCR's also had better systolic and diastolic function relative to control LCR's as well (Wisloff *et al.*, 2005).

Diseases like CVD are multifaceted and complex, and as a result, selection for a disease trait does not accurately reflect all of its complexities. By selecting rats based on a low running capacity (LCR's), selection is based on the mechanism of CVD and over time all of the complexities of the disease will be revealed (Koch and Britton, 2005).

1.5.3 Genetic contribution to aerobic and endurance capacity

The HCR's and LCR's were selected based on their intrinsic aerobic capacity. This model allows for the investigation of genes that determine aerobic capacity without

training and genes that determine the adaptational response to exercise and their interaction with the environment (Koch and Britton, 2001).

Aerobic capacity can be quantified by measuring VO_2max and in humans, VO_2max has a genetic component. Klissouras in 1971 demonstrated a 93% variability of VO_2max to be genetically determined in monozygotic and dizygotic male twins. Bouchard *et al.*(1999) demonstrated an adaptational response to exercise in 98 two-generation sedentary Caucasian families, where there was a significant contribution of genetics to the training response of VO_2max. The maximal heritable VO_2max value as a result of endurance training was found to be 47%. Endurance performance also has a genetic component. Perusse *et al.*, (2001) investigated the role of genetic factors in submaximal aerobic performance before and after a 20 week endurance training program. The response to endurance training showed a significant correlation between siblings and parent-offspring (genetic) and also between spouses (environmental factors). These resemblances were manifested as similar power outputs (PO) and oxygen consumption (VO_2) during submaximal exercise to exhaustion (Perusse *et al.*, 2001).

These studies cited above demonstrate the important role of genetics in determining aerobic capacity, trainability, and other parameters indicative of endurance performance (PO and VO_2). The HCR's and LCR's were selected based on their intrinsic endurance running capacity (without training) and with minimal environmental variation. Based on six generations of selection, the HCR's responded more strongly to selection than the LCR's (Koch and Britton, 2001). This method of selection should represent only the genetic component of endurance running capacity.

1.6 OBJECTIVES OF THE STUDY

Exercise intensity plays a major role in determining the fuels used for exercise; lipids are maximally oxidized at low to moderate exercise intensities and CHO are dominant at high exercise intensities. The regulation of the uptake of lipids into the muscle cell along with the mechanisms involved in the interaction of CHO and lipids during exercise are still unclear. A model for a high oxidative capacity and a low oxidative capacity associated with disease, may provide insights into CHO and lipid metabolism during exercise. In order to better understand the relationship between exercise intensity and fuel selection and the mechanisms involved, a unique rat model that was selected based on intrinsic running capacity was used to answer the following:

1. Does selection for high and low endurance running capacity also select for the ability to oxidize high and low amounts of lipids and CHO?
2. At what exercise intensity does maximal lipid and carbohydrate (CHO) oxidation occur at in a model for high and low endurance capacity?
3. Do LCR and HCR rats use the same proportions of lipid and CHO when exercised at the same percentage of their VO_2max?
4. Do the HCR's have higher activities of oxidative enzymes allowing them to oxidize greater absolute amounts of CHO and lipids at 50, 60, 70, and 80% VO_2max?
5. Does exercising at 60%VO_2max for 1 hr cause different changes in muscle metabolite levels in the HCR's and LCR's?

Since fuel selection patterns are conserved at submaximal exercise intensities in all mammals tested so far, it is expected that the HCR's and LCR's will follow this pattern despite differences in their aerobic capacity. Also, low exercise intensities will be dominated by lipid oxidation whereas high exercise intensities (>65%VO_2max) will be

dominated by CHO oxidation as seen in previous studies. It is also predicted that the HCR's will have a greater oxidative capacity and will oxidize more lipids and CHO than the LCR's, and will be able to accomplish this with skeletal muscle adaptations associated with an increased oxidative capacity.

CHAPTER 2: METHODS

2.1 ANIMALS

This study was approved by both the University of Michigan and McMaster University Animal Research Ethics Board (AREB). Experimental procedures were performed on 24 female rats bred from a genetically heterogeneous founder population of male and female rats (N:NIH). The rats were bred over 16 generations to be either high capacity runners (HCR's) or low capacity runners (LCR's) based on total distance run to exhaustion (in meters) using a progressive treadmill test (Koch and Britton, 2001). At 4-6 months of age the animals were transferred from the University to Michigan to McMaster University (12 HCR's and 12 LCR's) and allowed to recover from transport for a minimum of one week. Each group was given free access to a standardized rat diet. Both groups were housed two per cage, under a 12:12 hour light/dark photoperiod, and at 22°C. All rats were exercised at least once a week at 10 m/min for 10 minutes on a 10° incline if they were not participating in exercise tests.

2.2 INDIRECT CALORIMETRY

A flow-through respirometry system (Sable Systems, Henderson, NV) was used for measuring mass-specific oxygen consumption (VO_2) and carbon dioxide (VCO_2) production. Exercise measurements were made using a Plexiglas-enclosed motorized treadmill (Columbus Instruments, Columbus, OH). The flow rate of CO_2- and H_2O-free air through the treadmill chamber was regulated at 6000 ml/min (STPD) via a mass flow controller (Sable Systems, Henderson, NV). Drierite, and ascarite and soda lime were used to remove the H_2O and CO_2. Air was subsampled and flowed at ~250ml/min

through a Sable System FC-1B oxygen analyzer and a CA-2A carbon dioxide analyzer. The accuracy of the system was verified by burning methanol in the treadmill chamber as previously described (McClelland et al., 1998). This system was found to be accurate to within ±2%.

2.3 EXERCISE PROTOCOLS

Previously, Koch and Britton, (2001) determined distance to exhaustion through daily exercise trials for 5 days where treadmill speed was increased by 1 m min^{-1} every 2 minutes until the rat could no longer keep pace with the speed of the treadmill (Koch and Britton, 2001). The best daily run was used as the most representative distance to exhaustion measure. Maximal oxygen consumption (VO_2max) was determined for each individual rat using the protocol and criteria previously described (McClelland et al., 1998). Specifically, VO_2max was reached when the respiratory quotient (VCO_2/VO_2) was greater than 1, when there was no further change in VO_2 with an increase in speed and finally when the rat could not maintain position on the treadmill (McClelland et al., 1998). Running speeds corresponding to 50, 60, 70, and 80 % of VO_2max were determined for each individual using the relationship between running speed and VO_2 from the VO_2max trials. Animals were fasted overnight (12-16 hours) and were post-absorptive before O_2 and CO_2 was continuously monitored for 25 – 30 minutes at 50 and 60%max and 15-17 minutes for 70 and 80 %VO_2max at an angle of 10 degrees. All exercise intensity trials were done in a randomized fashion to minimize any potential influence of training on fuel selection patterns. The sample size for each group was n=12 for the HCR's and n=11 for the LCR's for all exercise intensities.

2.4 BLOOD SAMPLING AND TISSUE EXTRACTION

To understand the mechanisms underlying lipid and carbohydrate metabolism during submaximal exercise, blood and skeletal muscle tissue was sampled at rest (blood and muscle), during exercise (blood), and immediately after exercise (blood and muscle). Once the respirometry measurements were completed, many of the rats underwent jugular cannulation surgery at the Central Animal Facility at McMaster Hospital. A jugular vein catheter was inserted approximately 2 cm into the vein while the animal was under isofluorane anaesthetic (2.5 to 4%). The catheter was filled with heparinized saline (20 units/ml) and sealed with a sterile metal plug. The animal was given buprenophine analgesic (0.05mg/kg) before the initial surgical insult and in the morning on the day after surgery. Immediately after surgery, the animals were transferred back to their regular housing to recover for 2 days. After an overnight fast of approximately 10 hours, an extension was attached to the catheter and the rats were exercised at 60% of their VO_2max as previously determined by respirometry measurements. Rats were exercised for one hour and blood was sampled (0.3ml) from the catheter at rest, 5, 10, 15, 20, 30, 40, 50 and 60 minute time points. The samples were centrifuged immediately and stored in a -20 freezer. Once exercise was completed, the rats were given sodium pentabarbitol (0.2ml/100g) and the heart, liver, spleen and hindlimb tissue (medial and lateral gastrocnemius (MG, LG), soleus (SO), peroneus (P), tibialis anterior (TA), and extensor digitorum longus (EDL)) were immediately extracted, freeze-clamped, placed in liquid nitrogen and put into a -80 freezer until further analysis. Resting rats (those that underwent surgery but did not exercise) recovered for the same amount of time and were sampled for muscle tissue in the same manner. All skeletal muscle, heart, liver, and

spleen were powdered using a mortar and a pestle and stored at –80°C until further analysis.

2.5 ENZYME ASSAYS

Approximately 50 mg of frozen skeletal muscle tissue was homogenized for one minute using a glass homogenizer in an extraction buffer solution consisting of 20 mM Hepes, 1mM EDTA, and 0.1% Triton X 100 at a pH of 7.2. Samples were homogenized further using an ultrasonic cell disrupter (Microson; Framingdale, NY). A SpectraMAX Plus spectrophotometer (Molecular Devices; Sunnydale, California) set at 37°C was used to record the specific activities of lactate dehydrogenase (LDH), cytochrome oxidase (COX), β-hydroxyacyl CoA dehydrogenase (HOAD) and citrate synthase (CS). LDH was assayed at 340 nm with 50 mM of Imidazole (pH 7.4), 0.2mM sodium pyruvate, and 0.15 mM of NADH. CS was assayed at 412 nm with 0.1 mM 5,5-dithio-bis(2-nitrobenzoic acid) (DTNB), 0.3 mM acetyl-CoA, and 0.5 mM oxaloacetate. COX was assayed at 550 nm with 20mM Tris and reduced 50 M cytochrome c. HOAD was assayed at 340 nm with 0.1 mM acetoacetylCoA, and 0.15 mM NADH. Samples were measured in triplicates to ensure accuracy and were diluted if necessary to obtain optimal enzyme activity. Sample size was n=9 for the LCR's and n=10 for the HCR's for CS and COX for the soleus and medial gastrocnemius and only in the medial gastrocnemius for LDH. For LDH activity in the solues, sample size was n=5 and n=4 for the HCR's and LCR's respectively. For HOAD measurements the sample size was LCR=7 and HCR=8 for the soleus and LCR=6 and HCR=6 for the gastrocnemius.

2.6 METABOLITE MEASUREMENTS

Approximately 50mg of frozen skeletal muscle tissue was diluted in 600 ul of 6% perchloric acid, centrifuged for 10 minutes at 4°, and 360ul of the supernatant was removed for ATP and PCr assays and neutralized with 66ul of 3M K_2CO_3. Next the ATP/PCr samples were centrifuged at 4° C for 10 minutes and both assays were conducted on the same day. PCr was measured at 37°C with 1mM ADP, 5mM glucose, 2mM NAD^+, 0.020 U/µl of G-6PDH, 0.03U/ µl HK, and 1.65U/ µl CPK in 20mM Tris and 5mM $MgCl_2$. ATP was measured at 37°C with 5mM glucose, 2mM NAD^+, 0.020 U/µl of G-6PDH, and 6U/ µl HK in 20mM Tris and 5mM $MgCl_2$.

2.7 CALCULATIONS AND STATISTICS

Rates of VO_2 and VCO_2 were calculated using equations from Withers, (1977) and converted to their molar equivalents (MO_2 and MCO_2).

$$\mathbf{VO_2} = (FR\ (ml/min) \times 60 \times ((FeO_2 - FiO_2) - (VCO_2 \times FiO_2) / (1 - FiO_2) \quad (1)$$

$$\mathbf{VCO_2} = FR \times 60 \times (FeCO_2) \quad (2)$$

Where FR= flow rate, FiO_2 = concentration of O_2 entering the treadmill, FeO_2 = concentration of O_2 leaving treadmill, $FeCO_2$ = concentration of CO_2 leaving the treadmill. These values were converted to molar equivalents (MCO_2 and MO_2) by converting VCO_2 and VO_2 to L/min and dividing by 22.4 L/mole. This value was then divided by the weight of the rat.

Total lipid and CHO oxidation were calculated for each individual using indirect calorimetry equations (Frayn, 1983) assuming contribution of protein to overall metabolism was negligible:

VO_2 (l/min) = 0.746 carbohydrate + 2.03 fat (3)

VCO_2 (l/min) = 0.746 carbohydrate + 1.43 fat, (4)

rearranged to get:

Carbohydrate oxidation (g/min) = 4.55 VCO_2 – 3.21 VO_2 (5)

Fat oxidation (g/min) = 1.67 VO_2 – 1.67 VCO_2 (6)

Fractional contributions of lipid and CHO oxidation were calculated by dividing absolute rates by total MO_2. At 80%VO_2max, one rat had an RER over 1.0 and corresponding negative values for lipid oxidation at 10 minutes. At 50% VO_2max, 2 rats had negative values for CHO oxidation and percent CHO oxidation at 5 minutes of exercise and RER values of 0.7. We did not include these data points when calculating group averages. The time points chosen for Figure 6 did not include data that contained negative numbers. The data was analyzed by using a t-test or a 2-way repeated measures analysis of variance. Multiple comparisons were made using the Student-Newman-Keuls.

CHAPTER 3: RESULTS

3.1 DISTANCE TO EXHAUSTION, VO_2MAX, SPEED, AND BODYWEIGHT

After 16 generations of selection for endurance running capacity LCR and HCR rats revealed a 401% difference in total distance run to exhaustion. Maximum oxygen consumption (VO_2max) and the speed at which VO_2max was reached were determined for each individual from HCR and LCR. VO_2max was 1.3x higher (84.7±2.0 and 65.9±2.8 ml kg^{-1} min^{-1}; $P<0.001$) and speed at VO_2max was 1.6x higher in the HCR compared to LCR (39.3±1.1 and 24.9±0.9 m min^{-1}; $P<0.001$; Table 1). LCR were significantly heavier than HCR both at the beginning and at the end of the experiments. Both groups maintained steady body weight throughout the experiment (Table 1).

3.2 RESPIRATORY MEASUREMENTS

Rates of oxygen consumption (MO_2) and carbon dioxide production (MCO_2) appear in Figure 1. At all exercise intensities the HCR's had a significantly higher MO_2 and MCO_2 than the LCR's (at 50, 70 and 80%VO_2max, $P<0.001$ and at 60%VO_2max, $P=0.001$). Measurements among different levels of time revealed a significant difference in MCO_2 and MO_2 between the groups at all intensities except for 70%VO_2max ($P<0.001$ at 50, 60, and 80%VO_2max for MO_2 and MCO_2; $P>0.05$ for MCO_2 and MO_2 at 70%VO_2max). At all time points, there were differences between the HCR's and LCR's for MO_2 and MCO_2 at 60, 70, and 80%VO_2max (Fig. 1). Significant interactions between the HCR's and LCR's when time was considered occurred at 60%VO_2max ($P<0.001$ for MO_2, $P=0.016$ for MCO_2) and for MO_2 at 70%VO_2max ($P<0.001$). MO_2 for the LCR's

at 50, 60, and 70%VO_2max significantly decreased over time and significantly increased at 80%VO_2max (Fig.1A). The same pattern occurred in the HCR's at 50 and 70%VO_2max where MO_2 significantly decreased and at 80%VO_2max, MO_2 significantly increased (Fig. 1B). MCO_2 increased over time in both groups at 80%VO_2max and just in the HCR's at 70%VO_2max (Fig. 1C,D). MCO_2 decreased in both groups at 50% VO_2max and also at 60 and 70%VO_2max in the LCR's (Fig. C,D). The respiratory exchange ratio (RER) during exercise was not significantly different between the two groups at 60, 70 and 80%VO_2max and over time (60%VO_2max: $P>0.05$, $P>0.05$; 70%VO_2max: $P>0.05$, $P>0.05$; 80%VO_2max: $P>0.05$, $P>0.05$; Fig.2) except within the LCR's at 50%VO_2max; there was a significant difference found between 5 and 30 minutes ($P=0.04$). Average RER values for LCR and HCR over exercise at 60% VO_2max were 0.794 ± 0.004 and 0.807 ± 0.01. RER increased with exercise intensity to 0.84 ± 0.003 and 0.85 ± 0.003 at 70% VO_2max and to 0.907 ± 0.004 and 0.926 ± 0.004 at 80% VO_2max in the LCR's and HCR's, respectively.

3.3 EXERCISE INTENSITY

Target submaximal exercise intensities for this study were 50, 60, 70 and 80% VO_2max. Actual intensities reached by each individual appear in Figure 3. There were no significant differences between groups for the target exercise intensities of 60, 70, and 80%VO_2max. Measurements among different levels of time revealed a significant difference between groups at 50%VO_2max ($P<0.001$). Within the LCR's at 50%VO_2max, there was a significant decrease in %VO_2max as time progressed; at 5 minutes the LCR's were running at an average intensity of 62.2%VO_2max and the

intensity decreased to 50.2%VO_2max (Fig.3A). For the HCR's, the same trend was seen where the rats started running at an intensity of 57.3%VO_2max at 5 minutes and then decreased to 52.5%VO_2max at 30 minutes (Fig.3B). For both 50 and 70%VO_2max there were significant interactions found between the groups when time was considered ($P=0.001$ and $P=<0.001$ for 50 and 70%VO_2max respectively) however no specific time points were specified in the software. Average running speed (m/min) for the HCR's and LCR's at each exercise intensity is shown in Table 2. The HCR's had a significantly higher running speed at each intensity than the LCR's, and with each increase in intensity, the average running speed increased (Table 2).

3.4 TOTAL LIPID AND CHO OXIDATION RATES

Absolute CHO oxidation rates were significantly higher in HCR than the LCR at 70 and 80 %VO_2max ($P=0.008$ and $P<0.001$ respectively) and significant differences were found between the groups at all time points (Fig. 4C). At 50%VO_2max there were significant differences among the different levels of time when the two groups were considered ($P<0.05$). Within the LCR's, there was a significant difference between the first (5 minute) and last (30 minute) time point ($P<0.05$). For both groups of rats, CHO oxidation rates increased as exercise intensity increased (Fig. 4C,D). Lipid oxidation rates were higher in the HCR's than the LCR's at 50 and 70%VO_2max ($P<0.001$) and differences at each time point between the groups was found at each of these intensities (Fig. 4A). Similar rates of lipid oxidation were found at 60 and 80%VO_2max between the groups ($P>0.05$; Fig. 4A,B). Differences over time within the LCR's at 60%VO_2max were found between the first and last time points and second and last two time points

(Fig.4A). Maximal lipid oxidation rates occurred at 10 minutes in LCR (1392 ± 76 μmoles kg^{-1} min^{-1}), and 20 minutes in HCR (1621 ± 93 μmoles kg^{-1} min^{-1}) at 66.2 and 61.3% VO_2max respectively.

3.5 FRACTIONAL LIPID AND CHO USE

When expressed as a fraction of total energy expenditure, proportions of CHO used were similar between HCR and LCR at 50, 60, 70, and 80% VO_2 max (Fig. 5 C, D). Measurements among the different levels of time revealed significant differences at 50%VO_2max for both lipids and CHO ($P<0.05$) and at 80%VO_2max for lipids, between the HCR's and LCR's ($P<0.05$). Within the LCR's at 50%VO_2max there was a significant difference between the first and last three time points for percent CHO oxidation and a difference between the first and last time point for percent lipid oxidation (Fig.5A,C). Proportional lipid use was similar between groups except at 80%VO_2max where a difference was found at 10 minutes between the HCR's and LCR's (Fig.5A). Within the groups, there were significant differences between time points at 80%VO_2max. Specifically, the differences in the LCR's were seen between the first and last two time points and between the second and last time point for percent lipid oxidation (Fig.5A). In the HCR's, there were significant differences between the 5 minute and every other time point (Fig. 5B). The greatest percentage of lipids that were oxidized was at 50% VO_2 max for both groups (LCR: 89.3% ± 2.6; HCR: 79.9% ± 2.3) and the lowest percentage of CHO that were oxidized also occurred at this intensity (LCR: 10.6% ± 2.6; HCR: 19.9% ± 2.5).

3.6 LIPID AND CHO USE WITH EXERCISE INTENSITY

By including actual %VO_2max values for the target intensities of 50, 60, 70, and 80%VO_2max, absolute and fractional rates of lipid and CHO oxidation at 20 min (LCR: 51.8 ± 1.6 and 59.0 ± 3.0; HCR: 53.1 ± 1.5 and 61.3 ± 1.3) and 15 min (LCR: 67.3 ± 2.20 and 83.7 ± 2.2; HCR: 72.6 ± 2.4 and 86.3 ± 2.8) of exercise appear in Figure 6. The HCR's used significantly more lipids and CHO than the LCR's for all exercise intensities ($P<0.001$) except at 60%VO_2max for CHO (Fig. 6B). At each intensity, both groups of rats used the same proportions of CHO and lipids (Fig. 6 C,D) and with increasing intensity, it is clear that CHO become the dominant source of fuel to power exercise.

3.7 ENZYME ACTIVITIES

Enzyme activities of the soleus (COX, CS, HOAD, LDH) and gastrocnemius (COX, CS, HOAD) were determined in the HCR's and LCR's at rest and post exercise (Table 3). No significant differences were found between post exercise and resting data within the groups for either the soleus or medial gastrocnemius and therefore that data was combined for COX, CS, HOAD, and LDH (only medial gastrocnemius) to increases sample size. For LDH measurements in the soleus, significant differences in activity were found between rest and post exercise rats so only resting measurements were included in the results. For CS and COX, significant differences were found between the HCR's and LCR's in the soleus (CS: $P=0.007$; COX: $P=0.017$) and for HOAD, significant differences were found in the medial gastrocnemius ($P=0.004$); the HCR's had higher activities of those enzymes than the LCR's with an average value of 19.9 ±1 units g^{-1} wet weight compared to 14.8 ±1.4 units g^{-1} wet weight for CS in the soleus. COX

activities were 11.6 ±0.7 units g^{-1} wet weight versus 8.4 ±1.2 units g^{-1} wet weight and HOAD activities were 3.1 ±0.3 units g^{-1} wet weight versus 1.7±0.2 units g^{-1} wet weight for the soleus and medial gastrocnemius respectively. No other differences were found between the HCR's and LCR's (Table 3).

3.8 METABOLITE CONCENTRATIONS

Concentrations of ATP and PCr were measured in various hindlimb muscles in the HCR's and LCR's and appear in Table 4. In the EDL, there was a significant difference in ATP and PCr concentrations between pre and post exercise samples in both the HCR's and LCR's; PCr and ATP concentrations significantly decreased from rest (PCr: HCR: 10.5±1 to 6.1±1 μmol g^{-1} wet tissue, LCR: 11.3±3 to 4.7±0.7 μmol g^{-1} wet tissue; ATP: HCR: 4.8± 0.4 to 3.3± 0.5 μmol g^{-1} wet tissue, LCR: 4.3± 0.4 to 3.2±0.3 μmol g^{-1} wet tissue). There were no other differences between the HCR's and LCR's or differences within the groups at rest and immediately after exercise.

Table 1: Distance to Exhaustion (metres), maximum aerobic capacity (VO_2max), speed at VO_2max, and body mass (Mb) for the HCR's and LCR's.

Table 1 shows meters run to exhaustion, VO_2max in millimeters per gram per hour (ml g^{-1} h^{-1}) and millimeters per kilogram per minute (ml kg^{-1} min^{-1}), speed at VO_2max in metres per minute (m min^{-1}), and body mass of the LCR's and HCR's in grams (g) at the beginning and end of the experiment. Sample size is n=12 for each group. The * indicates significantly different from LCR's and the # indicates significantly different from the HCR's. Values are ± SEM.

	Meters to exhaustion	VO_2max (ml g^{-1} h^{-1})	VO_2max (ml kg^{-1} min^{-1})	Speed (m min^{-1})	Mb beginning (g)	Mb end (g)
HCR	1511 ± 6*	5.08 ± 0.12*	84.7 ± 2.0*	39.3 ± 1.1*	221 ± 7	221 ± 6
LCR	377 ± 2	3.95 ± 0.17	65.9 ± 2.8	24.9 ± 0.9	269 ± 7 #	280 ± 6 #
HC/LC	+ 4.01	+ 1.29	+ 1.29	+ 1.58	0.82	0.79

Table 2. Treadmill running speed (m/min) of the HCR's and LCR's at 50, 60, 70 and 80%VO_2max.

Table 2 shows the average running speed (m/min) of the HCR's and LCR's for each relative exercise intensity (%VO_2max). The * indicates significantly different from the HCR's and values are ± SEM.

	Relative exercise intensity (%VO_2max)			
Speed (m min^{-1})	**50%**	**60%**	**70%**	**80%**
HCR	13.4±0.95	18.5±0.86	23.8±0.87	29.0±0.89
LCR	9.9±0.91*	12.9±0.74*	15.9±0.62*	18.9±0.59*

Figure 1. Rates of oxygen consumption (MO_2) and carbon dioxide production (MCO_2) for the LCR's and HCR's at 50, 60, 70, and 80% VO_2max.

Graph A and B show MO_2 and graph C and D show MCO_2 for the LCR's (n=11) and HCR's (n=12) respectively. The * indicates significantly different from the HCR's. The **o** indicates significant differences at all time points between the HCR's and LCR's. The # and $ indicates significantly different from 5 and 10 minutes within the LCR's and & and ^ indicates significantly different from 5 and 10 minutes within the HCR's. Values are all ± SEM.

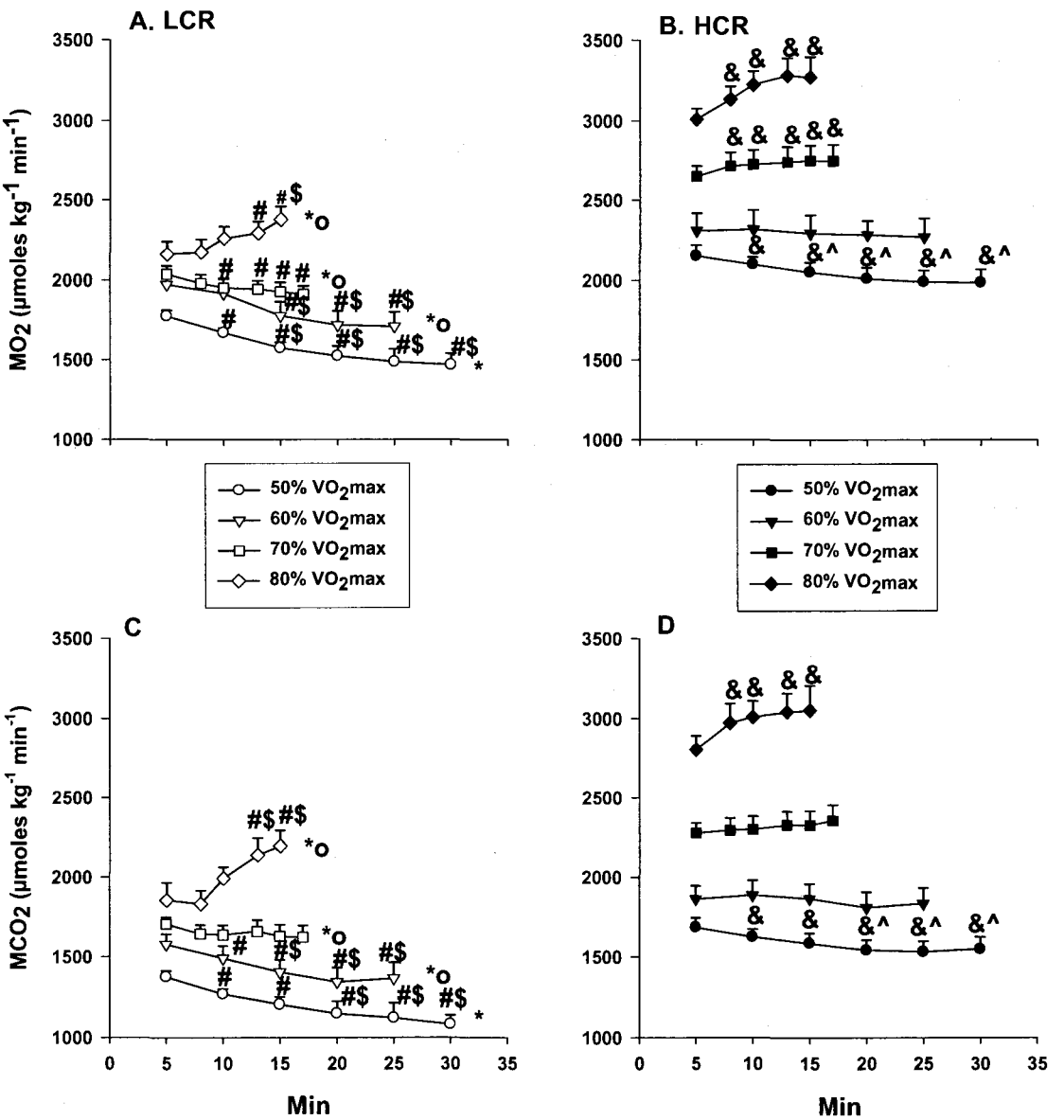
A. LCR
B. HCR
C
D
MO2 (µmoles kg-1 min-1)
MCO2 (µmoles kg-1 min-1)
Min
50% VO2max
60% VO2max
70% VO2max
80% VO2max

Figure 2. Respiratory exchange ratio (RER) for the LCR's and HCR's at 50, 60, 70, and 80% VO_2max.

Graph A and B show the mean RER's for the LCR's and HCR's respectively. Sample size is n=12 for the HCR's, and n=11 for the LCR's. The # indicates significantly different from 5 minutes within the LCR's and values are ± SEM.

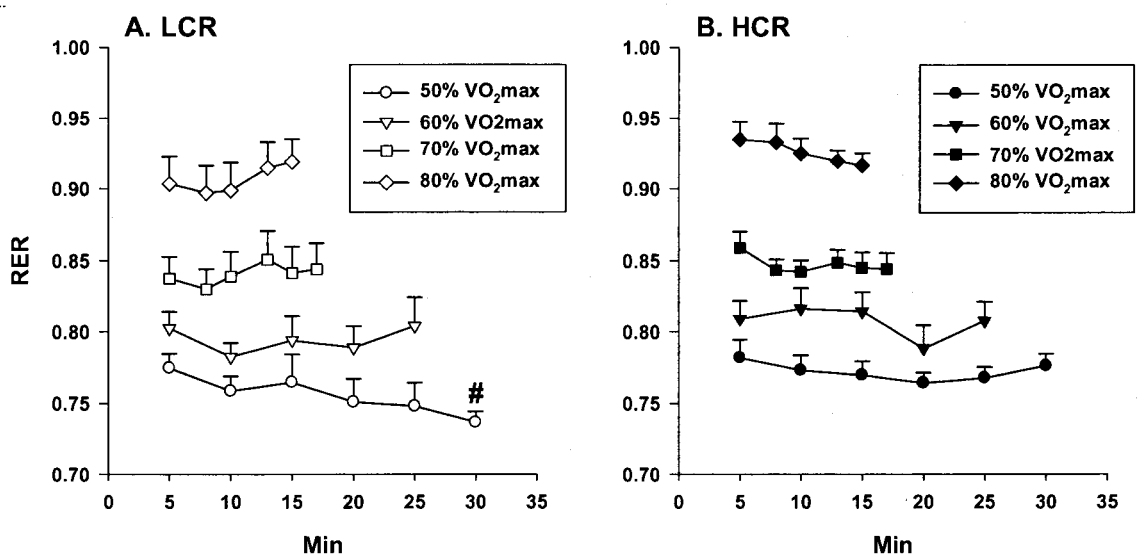
A. LCR
B. HCR
RER
Min
50% VO2max
60% VO2max
70% VO2max
80% VO2max
#

Figure 3. Actual %VO_2max values for all intended exercise intensities over time for the LCR's and HCR's.

Graph A and B represent the actual mean %VO_2max that the LCR's and HCR's ran at, respectively. Sample sizes are n=12 for HCR's and n=11 for LCR's. The #, $, and + indicates significantly different from 5, 10, and 15 min within the LCR's and the & indicates significantly different from 5 min within the HCR's. All values are ± SEM.

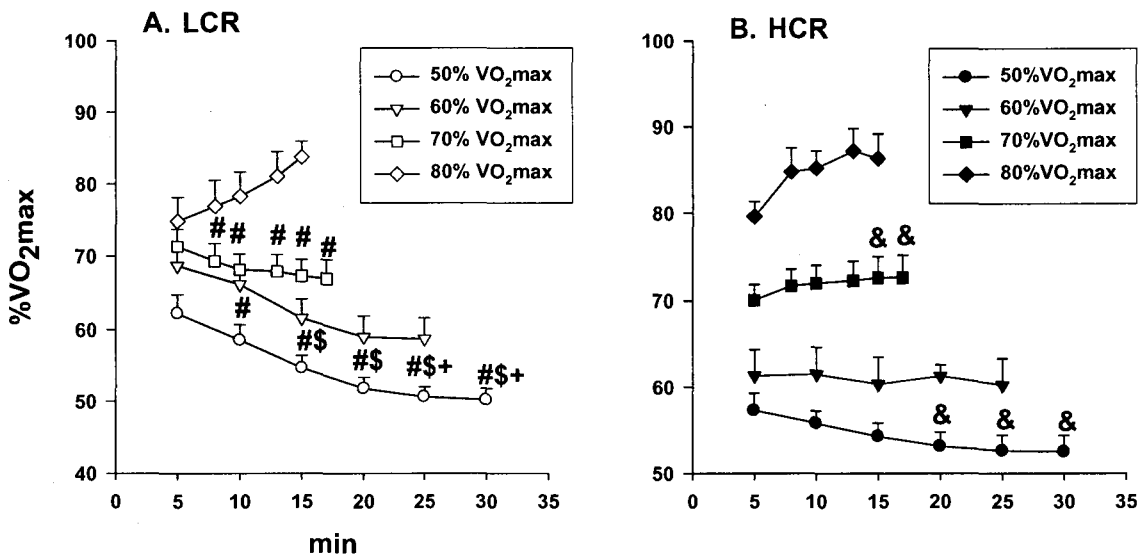
A. LCR
B. HCR
%VO2max
min
50% VO2max
60% VO2max
70% VO2max
80% VO2max
50%VO2max
60%VO2max
70%VO2max
80%VO2max
100
90
80
70
60
50
40
0
5
10
15
20
25
30
35
#
#$
#$+
&

Figure 4. Rates of total lipid and CHO oxidation in the LCR's and HCR's at 50, 60, 70 and 80% VO_2max.

Graphs A and B represent total lipid oxidation and graphs C and D represent total CHO oxidation in the LCR's and HCR's. Sample size is n=12 for HCR's and n=11 for LCR's. * indicates significantly different from the HCR's. The o indicates differences at all time points between the HCR's and LCR's. The " indicates differences between the HCR's and LCR's at a specific time point. The # and $ indicates significantly different from 5 and 10 min for the LCR's. All values are ± SEM.

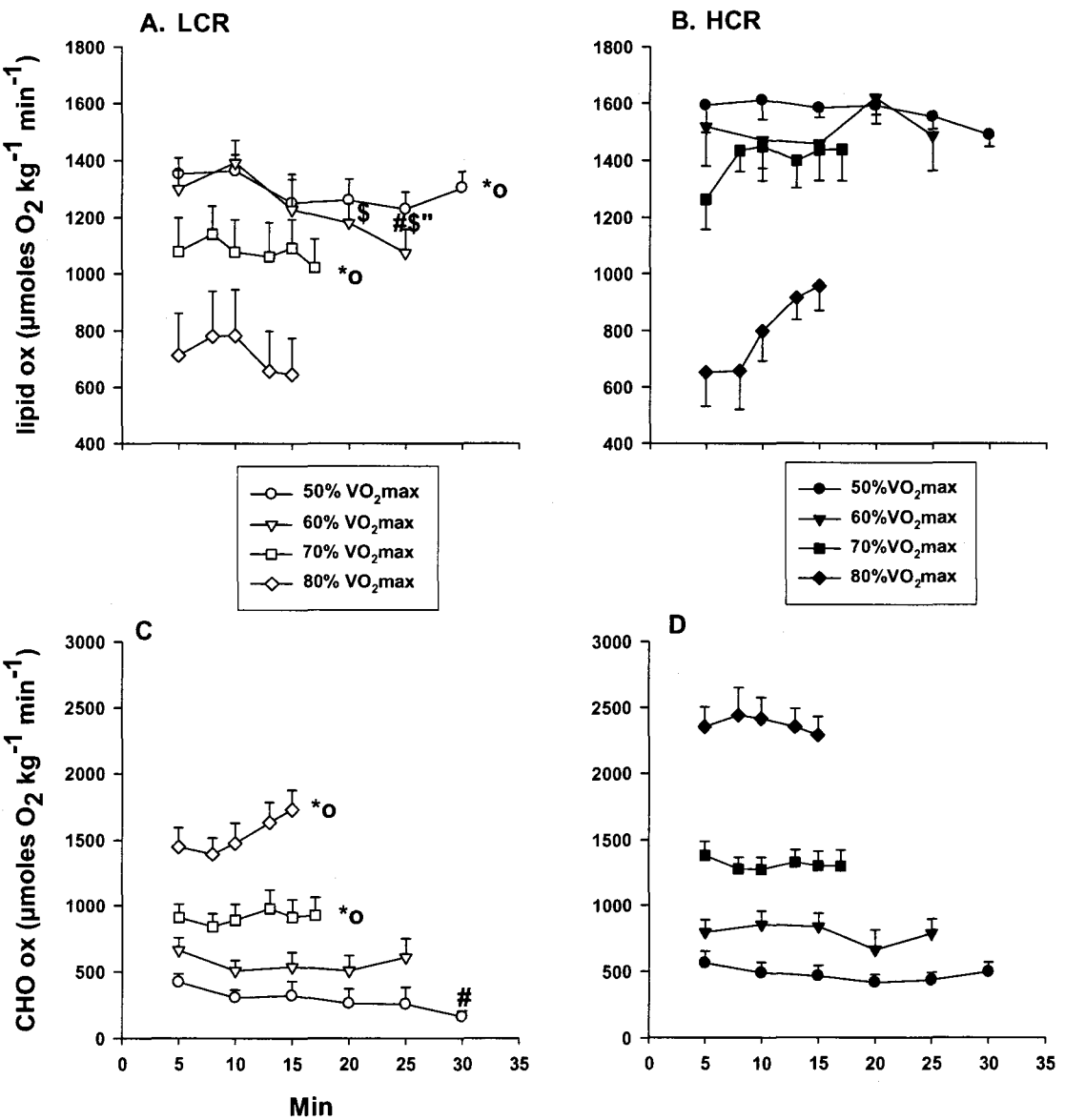
A. LCR
B. HCR
C
D
lipid ox (µmoles O2 kg-1 min-1)
CHO ox (µmoles O2 kg-1 min-1)
Min
50% VO2max
60% VO2max
70% VO2max
80% VO2max
50%VO2max
60%VO2max
70%VO2max
80%VO2max
*o
$
#$"
#

Figure 5: Percent lipid and CHO oxidation rates in the LCR's and HCR's at 50, 60, 70 and 80% VO_2max.

Graphs A and B represent percent lipid oxidation and graphs C and D represent percent CHO oxidation for the LCR's and HCR's. Sample size is n=12 for HCR's and n=11 for LCR's. The * indicates significantly different from the HCR's. The " indicates a difference between HCR's and LCR's at a specific time point. The # and $ indicates significantly different from 5 and 10 minutes for the LCR's and & indicates significantly different from 5 minutes for the HCR's. All values are ± SEM

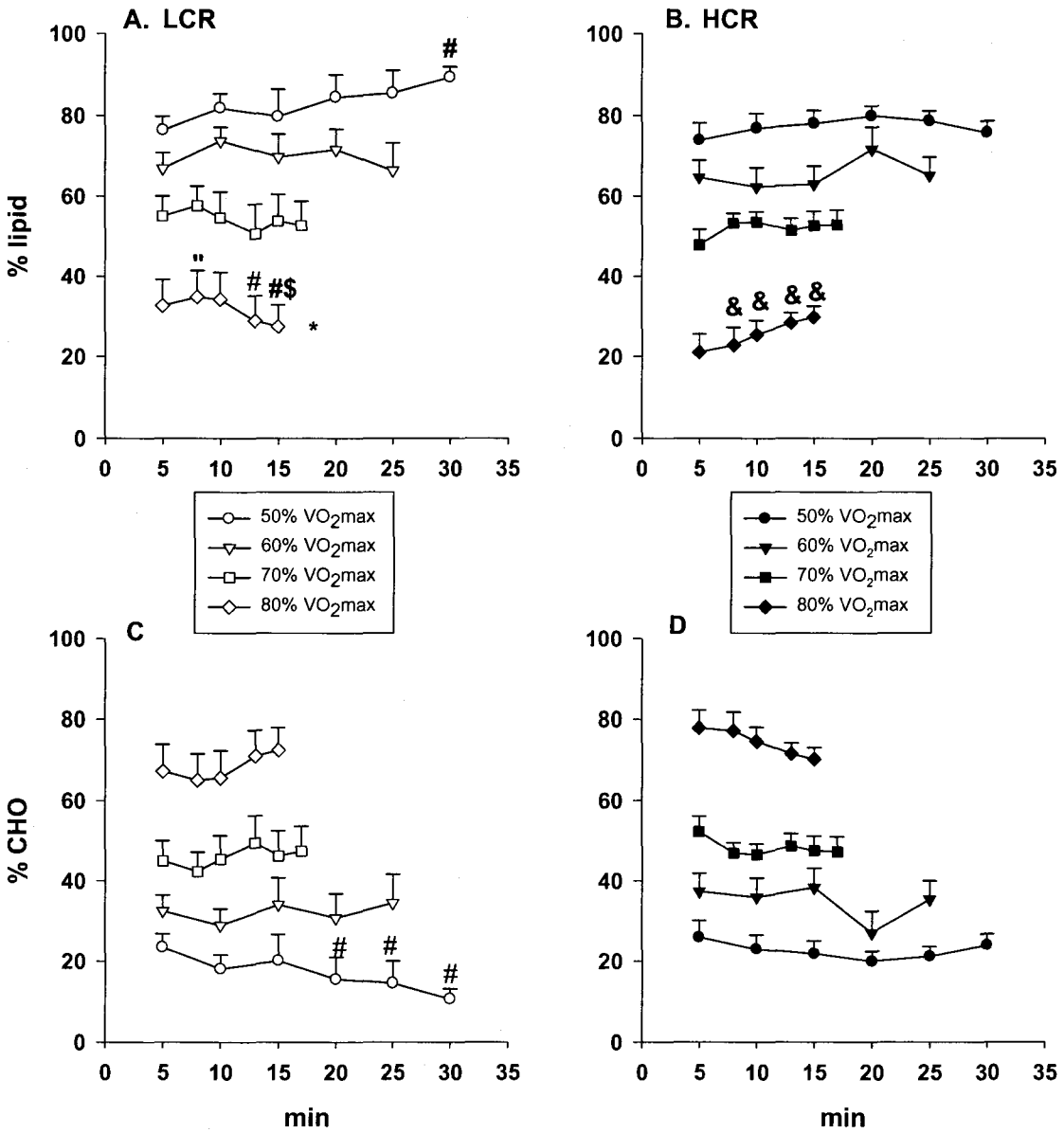
A. LCR
B. HCR
C
D
% lipid
% CHO
min
50% VO2max
60% VO2max
70% VO2max
80% VO2max

Figure 6: Absolute and relative lipid and CHO oxidation rates versus %VO_2max in the LCR's and HCR's.

Graphs A and B show total rates of lipid and CHO oxidation versus %VO_2max and, graphs C and D show percent lipid and CHO versus %VO_2max in the HCR's and LCR's at approximately 50, 60, 70, and 80%VO_2max. Twenty minute and fifteen minute time points were chosen for 50 and 60%VO_2max, and 70 and 80%VO_2max, respectively. For percent total fuel oxidation, values are expressed relative to total VO_2 (100%). The * indicates significantly different from the HCR's and values are ± SEM for %VO_2max (horizontal error bars) and % fuel oxidation (vertical error bars).

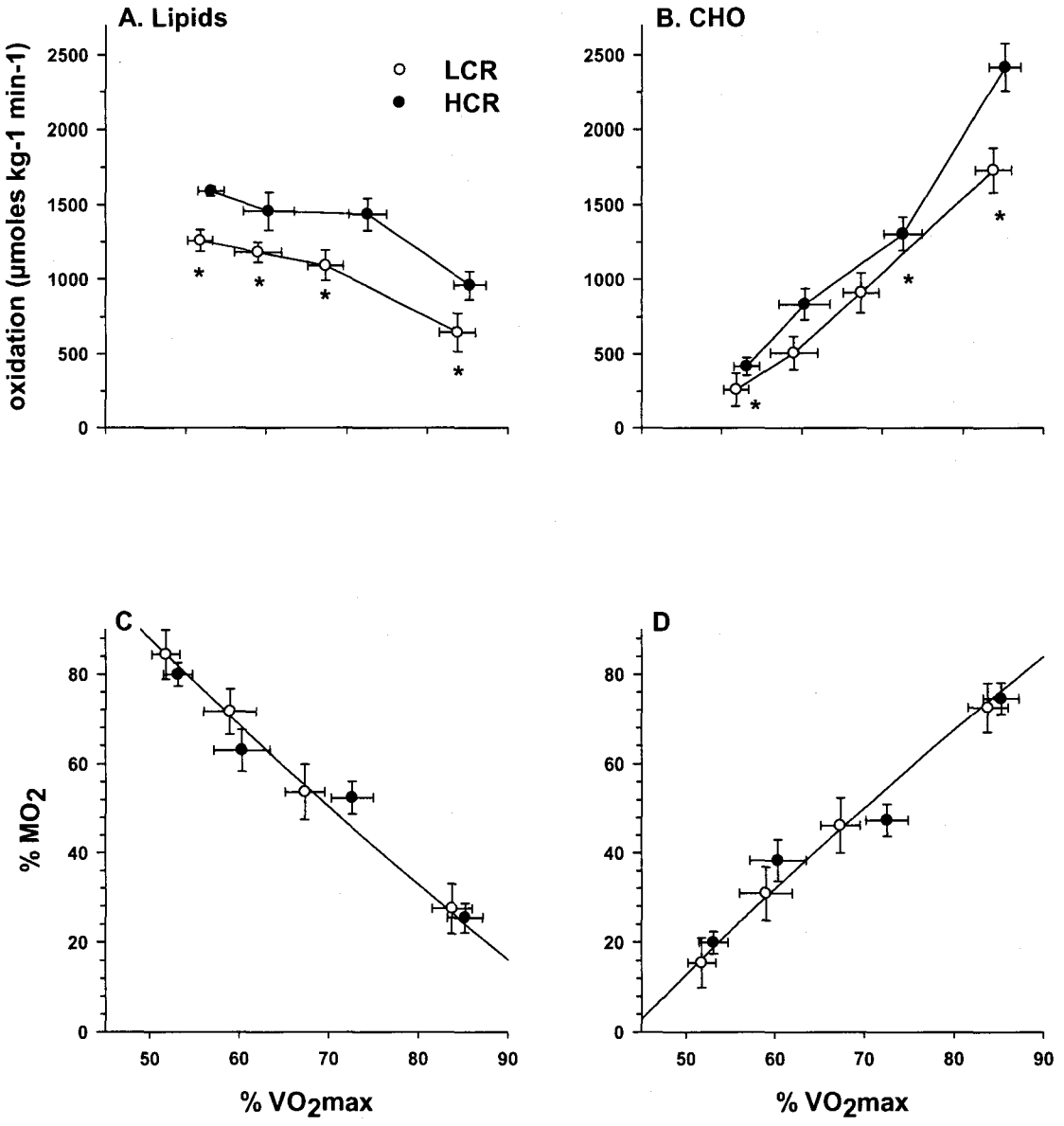
A. Lipids
LCR
HCR
oxidation (µmoles kg-1 min-1)
2500
2000
1500
1000
500
0
B. CHO
C
% MO2
% VO2max
D
% VO2max
50
60
70
80
90
20
40

Table 3: Enzyme activities of the soleus (SO) and medial gastrocnemius (MG) in HCR's and LCR's at rest/post exercise.

Table 3 shows COX, CS, HOAD and LDH activities for the soleus and medial gastrocnemius in the HCR's and LCR's. Activities are in U g^{-1} wet weight where a unit is μmol min^{-1}. The * indicates significantly different from the HCR's and values are ± SEM.

	COX		CS		HOAD		LDH	
	HCR	**LCR**	**HCR**	**LCR**	**HCR**	**LCR**	**HCR**	**LCR**
SO	11.6± 0.7	8.3± 1.2*	19.9± 1.0	14.8± 1.4*	3.5± 0.5	3.4± 0.4	432.1± 97.8	342.5± 66.7
MG	9.6± 0.8	9.7± 1.0	14.6± 1.2	10.2± 1.8	3.1± 0.3	1.7± 0.2*	92.6± 4.6	102.1± 7.1

Table 4: Creatine Phosphate (CrP) and ATP concentrations (g^{-1} wet weight) in hindlimb muscle of the HCR's and LCR's.

Table 4 shows CrP and ATP concentrations in grams per wet weight of tissue (g^{-1} wet weight) in the medial gastrocnemius (MG), lateral gastrocnemius (LG), Soleus (SO), tibialis anterior (TA) and extensor digitorum longus (EDL) of the HCR's and LCR's at rest and post exercise. The * indicates significant difference between rest and post exercise and values are ± SEM.

	PCr				ATP			
	HCR		LCR		HCR		LCR	
	Pre	post	pre	Post	Pre	Post	pre	post
MG	18.9 ±3	10.9 ±2	18.2 ±0.7	15.1 ±2	5.3 ±0.4	5.0 ±0.4	5.6 ±0.2	4.7 ±4.0
LG	11.4 ±0.3	10.2 ±4	12.9 ±1	12.6 ±2	3.9 ±1	3.9 ±1	3 ±0.6	4.6 ±0.6
SO	8.3 ±4	8.4 ±2	7.9 ±3	10.3 ±2	3.1 ±0.9	3.1 ±0.9	3.6 ±0.9	1.2 ±0.5
TA	6.1 ±2	9.5 ±0.7	10.5 ±1	9.3 ±1	5.2 ±1	5.2 ±1	4.4 ±0.8	5 ±0.7
EDL	10.5 ±1	6.1 ±1*	11.3 ±3	4.7 ±0.7*	4.8 ±0.4*	3.3 ±0.5*	4.3 ±0.4	3.2 ±0.3*

CHAPTER 4: DISCUSSION

The HCR's and LCR's used the same proportions of lipids (except at 80%VO_2max) and CHO (50, 60, 70, and 80%VO_2max) at each relative submaximal exercise intensity tested despite a 1.3 times higher VO_2max and 401% greater distance to exhaustion in the HCR's (Fig 5,6; Table 1). Also, the HCR's have a higher oxidative capacity than the LCR's with greater overall absolute oxidation rates for CHO and lipids (Fig 4,6) and greater mitochondrial enzyme activity (CS and COX in soleus; HOAD in gastrocnemius; Table 3). The maximal rate of lipid oxidation was found to be at 60%VO_2max for both groups of rats (Fig 4A,B). The focus of the discussion will be on the mechanisms and factors involved in explaining CHO and lipid use during exercise intensity along with the methods used in this study, genetic considerations, and possible future directions.

4.1 INDIRECT CALORIMETRY: ASSUMPTIONS AND VALIDITY

Indirect calorimetry was used in this study to measure whole-body rates of gas exchange (O_2 consumption and CO_2 production), at the level of the lungs. In using this technique there are a few disadvantages and assumptions that are made that are to be taken into consideration. For instance, small differences in calculations can greatly affect CHO oxidation rates by up to 6% (Jeukendrup and Wallis, 2004; Frayn, 1983). Also, many equations that have been developed to calculate oxidation rates are based on resting conditions where glucose is the representative CHO (Jeukendrup and Wallis, 2004). During moderate to heavy exercise, glycogen is used for energy which can result in oxidation rates that are lower than glucose (about 10%). Also, because O_2 and CO_2 are

measured at the level of the lung there is the potential that CO_2 can be overestimated, particularly at high exercise intensities. This is true because at those intensities, H^+ ions accumulate and are buffered by HCO_3- resulting in an excess amount of CO_2 production and over-estimation of CHO oxidation. Also, by using this technique, it is assumed that all of the CO_2 and O_2 are accounted for when in reality, there are other metabolic processes that can contribute to these values including lipogenesis and gluconeogenesis (Jeukendrup and Wallis, 2004).

Aside from these assumptions, indirect calorimetry has been shown to be an reasonably accurate way of quantifying CO_2 production and O_2 consumption. For example, Romijn *et al.*, (1992) compared the $^{13}C/^{12}C$ breath method and indirect calorimetry in quantifying lipid and CHO oxidation rates during high intensity exercise (80-85%VO_2max). It was found that both methods yielded similar values at rest and during exercise (Romijn *et al.*, 1992). Along with this study, indirect calorimetry continues to be a widely used technique in physiology.

4.2 FUEL SELECTION DURING SUBMAXIMAL EXERCISE INTENSITIES

4.2.1 Maximal lipid oxidation

The maximal rate of lipid oxidation in both groups of rats occurred at 60%VO_2max at 10 minutes in the LCR's (1392.1 ± 76 μmoles $kg^{-1}min^{-1}$) and at 20 minutes in the HCR's (1620.8 ± 93 μmoles $kg^{-1}min^{-1}$; Fig.4A, B). Goats and dogs oxidized fat maximally at 40%VO_2max (Roberts *et al.*, 1996) and maximal fat oxidation in humans has been shown to occur between 55 and 65% VO_2max depending on factors such as training status and gender (Achten and Jeukendrup 2003; Romijn *et al.* 1993;

Stisen *et al.*, 2006). At rest, small mammals are already at 30-40% of their VO_2max because they have a lower aerobic scope. Romijn *et al.* 1993 determined that endurance-trained cyclists oxidize fat maximally at 65%VO_2max and declined when exercise intensity increased to 85%VO_2max. Similarly, moderately trained men have been shown to oxidize fat maximally at 63%VO_2max (Achten and Jeukendrup 2003) and untrained and trained women maximally oxidize fat at approximately 55%VO_2max (Stisen *et al.*, 2006). In this study, the highest rate of lipid oxidation occurs at 60%VO_2max in both groups of rats however at 50%VO_2max, lipid oxidation rates are consistently high (see Fig4A, B). In general, moderate exercise intensities ranging from 50 to 60%VO_2max in the HCR's and LCR's seem to be the upper limit to fat oxidation despite their differences in aerobic capacity.

4.2.2 Absolute rates of oxidation

The results show that the HCR's oxidize a greater amount of CHO and lipids at each submaximal exercise intensity despite the differences not always being statistically significant (see Fig.4). In comparison to McClelland *et al.*, (1998), total CHO oxidation rates were similar at 60% and 80%VO_2max. For example, the highest CHO oxidation rates at 60%VO_2max for sea level (SL) rats was 875 ± 74 μmol $O_2\,kg^{-1}min^{-1}$ and for high altitude acclimated (HA) rats was 682 ± 86 μmol $O_2\,kg^{-1}min^{-1}$ in the McClelland *et al.* (1998) study; in the present study, the highest CHO oxidation rates in the HCR's was at 851 ± 104 μmol $O_2\,kg^{-1}min^{-1}$ and for the LCR's was 660 ± 93 μmol $O_2\,kg^{-1}min^{-1}$. These rates are similar between the two studies when comparing the groups with the higher VO_2max (HCR's and SL rats) and the groups with the lower VO_2max (LCR's and HA

rats). Similar rates of CHO oxidation can be seen at 80%VO_2max when comparing the two studies as well. For total lipid oxidation rates, published values from McClelland *et al.*, (1999) reveal that at 60%VO_2max, the values are similar between the high VO_2max groups (HCR's and SL) and low VO_2max groups (LCR's and HA). At 80%VO_2max, absolute lipid oxidation rates are generally lower in the present study compared to the values in McClelland *et al.*, (1999); HCR: 959 ± 87 μmol O_2 $kg^{-1}min^{-1}$ and SL: 1466 ± 102 μmol O_2 $kg^{-1}min^{-1}$; LCR: 783 ± 162 μmol O_2 $kg^{-1}min^{-1}$ and HA: 985 ± 76 μmol O_2 $kg^{-1}min^{-1}$. Many of the values obtained for this study are similar to McClelland *et al's* (1998, 1999); but within this study, not all of the values are statistically different. One explanation could be that the exercise trials may not have been long enough to see the differences between the HCR's and LCR's, especially at higher intensities. At 80% VO_2max, total lipid oxidation shows an upward trend (higher oxidation rates) in the HCR's and a downward trend (lower oxidation rates) in the LCR's possibly due to increased in relative exercise intensity (Fig.3A, 4A,B). This may also apply at 60%VO_2max where the LCR's show a decrease in overall lipid oxidation with time; the last time point (25 minutes) was shown to be significantly different between the groups (see Fig. 4A, B). The decreased absolute rates of lipid oxidation also reflect decreased MO_2 (Fig. 1A,B) and %VO_2 for lipids did not change over time (Fig. 5A,B). For absolute rates of CHO oxidation, the higher exercise intensities (70 and 80%VO_2max) elicited a significant difference between the HCR's and LCR's, with the HCR's oxidizing a greater amount of CHO than the LCR's (Fig.4C, D). Over time at 50%VO_2max, there are significant differences between the HCR's and LCR's at each time point indicating that the HCR's are in fact oxidizing a greater amount of CHO than the LCR's when time

is considered (Fig.4C). Although there was a trend at 60%VO_2max for the HCR's oxidizing a greater amount of CHO than the LCR's, it was not statistically different (Fig.4C). As with total lipid oxidation, decreased absolute rates of CHO oxidation reflect decreased MO_2 (Fig. 1A,B) and %VO_2 did not change over time for CHO (Fig. 5C,D). Since these two groups of rats have different rates of CHO and lipid oxidation, they provide an ideal model to examine the mechanisms responsible for regulating fuel use.

4.2.3 Fuel selection during relative exercise intensities

The HCR's and LCR's, despite significant differences in aerobic capacity (Table 1), use the same proportions of lipids (except at 80%VO_2max) and CHO when exercising at the same percentage of their VO_2max (Fig 5,6). Fifteen and twenty minute time points were chosen for Figure 6 in order to ensure that a steady state of oxidation had been reached since MO_2 for example, changed over time (Fig.1). At those chosen time points, similar proportions of lipids and CHO were used by the HCR's and LCR's (Fig.6). As mentioned in the introduction (1.2.2), this pattern exists in all mammals and conditions studied so far. Trained and untrained rats oxidize the same percentage of glucose when exercising at the same work rate (Brooks and Donovan, 1983). Dogs and goats use the same proportions of lipids and carbohydrates when exercising at 40, 60, and 85% of their VO_2max despite the dog having twice the aerobic capacity than the goat (Roberts *et al.*, 1996). Even altitude does not alter this pattern of fuel selection since high altitude and sea level rats oxidize the same proportions of lipids and carbohydrates at 60 and 80% of their VO_2max (McClelland *et al.*, 1998, 1999). Within this pattern of fuel selection, it is

also evident that with increasing exercise intensity, CHO oxidation rates increase and lipid oxidation rates decrease accordingly (see Fig.4, 6).

Although, this pattern of fuel selection provides an important framework for future studies, there are some examples of deviations from this pattern. For instance, Friedlander *et al.*, (1998) showed that women increased their total lipid use after training when they exercised at the same %VO_2max pretraining, which is in contrast to previous findings in men, by the same authors (Friedlander *et al.,* 1997). As a result, trained and untrained women use different total amounts of lipids when exercising at the same relative exercise intensity.

Diet also has an effect on the fuel patterns observed during relative exercise intensities. For example, Helge *et al.*, 2001 demonstrated that a high fat diet increased whole-body fat utilization at 68%VO_2max, compared to a CHO-rich diet, and resulted in a lower RER value of 0.86 at this exercise intensity. Increased fat oxidation was due to increased fatty acid oxidation and also from the oxidation of very low density lipoprotein-triacylglycerol. Decreased CHO oxidation was determined to be a result of glycogen sparing since blood glucose utilization was similar between the high-fat and CHO-rich diets (Helge *et al.*, 2001). This study suggests that a high fat diet alters fuel utilization by allowing more lipids to be oxidized at an intensity that is typically dominated by CHO oxidation.

4.3 MECHANISMS INVOLVED IN FUEL SELECTION DURING EXERCISE

The mechanisms responsible for the patterns of fuel selection mentioned above are currently unclear however there are a few hypotheses, including the regulation of the

interaction between CHO and lipid metabolism, enzyme and transporter regulation, and fiber type and recruitment patterns (for review see McClelland, 2004).

4.3.1 Fat oxidation is limited at high exercise intensities

As seen in this and many other exercise studies using mammals, fat oxidation is at a maximum at moderate exercise intensities and either does not change or decreases with a further increase in exercise intensity. The mechanism responsible for this decrease is currently unknown however there are studies that have provided insight into fat oxidation and it's limitations during high intensity exercise. Using well-trained cyclists, Romijn *et al.* (1993) demonstrated that as exercise intensity increased from 65 and 85%VO_2max, the rate of appearance of FFA's (mobilization) decreased and glucose uptake and glycogen utilization increased. In a subsequent study, Romijn *et al.* (1995) showed that maintaining FFA availability at 85%VO_2max, by using lipid/heparin infusions, did not fully restore fat oxidation rates suggesting that there is another mechanism (other than fat availability) that is responsible for decreased fat oxidation rates at this intensity. Furthermore, Sidossis *et al.*, (1997) showed that there is an inhibition of long chained fatty acid's (LCFA's), not medium chained fatty acids, going into the mitochondria that are probably contributing to the overall decrease in lipid oxidation at an exercise intensity of 80%VO_2max. In the present study, the HCR's and LCR's may be unable to oxidize more fat at higher exercise intensities because of FA transport or uptake limitations and/or the inability of the muscle to use lipids for energy. Regardless, the mechanisms involved need to be elucidated in order to understand why fat oxidation decreases during high intensity exercise, some of which are discussed below.

4.3.2 CPT-I and Malonyl-CoA

CPT-I is an important regulatory enzyme, controlling the flux of fatty acid through β-oxidation by catalyzing the reaction between fatty acyl CoA and free carnitine to form long-chain acylcarnitines. An understanding of its exact role in fatty acid metabolism and how it is regulated may be crucial in understanding some of the unknowns in fat metabolism during exercise. CPT-I is allosterically regulated by malonyl-CoA (M-CoA) and many studies have shown that an increase in M-CoA results in the inhibition of CPT-I (Kerner and Hoppel, 2000). Recently however there is evidence to suggest that M-CoA may not be the only factor responsible for regulating CPT-I activity and as a result, the relationship between CPT-I and M-CoA in both rats and humans is still unclear.

In rats, Winder in 1989 showed that 30 minutes of moderate treadmill exercise (21m/min at a 15% grade) caused a significant decrease in M-CoA concentration in the gastrocnemius muscle. This suggests an important role for M-CoA in allowing fatty acid oxidation to occur, possible through the lack of inhibition on CPT-I activity. In contrast, Odland et al. (1996) found that M-CoA levels in humans, were unaffected by 70 minutes of low to moderate intensity exercise (10 minutes at 40%VO_2max and 60 minutes at 65-70%VO_2max) but were decreased in rat muscle, even though fat oxidation rates increased. This study suggests that M-CoA may not be the principle factor in the regulation of fat metabolism during exercise in humans.

In another study, CPT- I inhibition by M-CoA was found to be similar between humans and rats. Bezaire et al. (2004) found that M-CoA levels declined allowing fat oxidation to occur through CPT-I during exercise at 65%VO_2max in human and rat

mitochondrial fractions. But a more recent study in humans by Holloway *et al.*, (2006) found that moderate intensity exercise (60%VO_2max) past 30 minutes (up until 120 minutes) decreased the sensitivity of CPT-I to M-CoA as whole body and palmitate mitochondrial rates of oxidation increased. This suggests that there is another mechanism allowing fat oxidation to occur other than the M-CoA and CPT-I mechanism.

One potential explanation for these findings may be that M-CoA inhibition of CPT-I and fatty acid oxidation may be fiber-type specific (Kim *et al.*, 2002). Kim *et al.*, (2002) showed that in rat white muscle, M-CoA inhibited palmitate oxidation by 75-90% but in red muscle, it was only inhibited by 35-54%. From this study it seems that there is evidence of M-CoA insensitive CPT-I activity in rat red muscle (Kim *et al.*, 2002).

From these studies in rats and humans, it seems that M-CoA does affect CPT-I in some instances during exercise and that species differences exist. Clearly, there are other mechanisms involved in regulating CPT-I and fat oxidation other than M-CoA.

4.3.3 Free carnitine and CPT-I activity

Carnitine has been investigated for its role in fatty acid oxidation during exercise because it has been shown to decrease in concentration with FA oxidation (Chien *et al.*, 2000; van Loon *et al.*, 2001). It is an important substrate for CPT-I in transporting LCFA's across the inner mitochondrial membrane and also acts as a buffer for excess acetyl-CoA that accumulates during conditions of high glycolytic flux when it is not being used by the Kreb's cycle (Stephens *et al.*, 2007). The concentration of carnitine decreases during high intensity exercise and as a result, there is less available for CPT-I to use for transporting long chain acyl CoA's into the mitochondrial matrix to be oxidized

(van Loon *et al.*, 2001; Stephens *et al.*, 2006; Stephens *et al.*, 2007 in press). As a result, it may play an important role in regulating CPT-I activity in situations of high energy utilization.

In humans and rats, a decrease in muscle carnitine concentration is associated with a decrease in fat oxidation. van Loon *et al.*, (2001) studied male cyclists at 40, 55, and 75%VO_2max in order to determine if carnitine limits CPT-I activity and fat oxidation at these intensities. It was found that at 75%VO_2max, fat oxidation along with the carnitine pool decreased, and CHO oxidation increased. The effect on CPT-I activity was not clearly determined but there is a possibility that the decrease in carnitine that was seen may have resulted in limited CPT-I activity (van Loon, 2001).

In another study, Stephens *et al.* (2006) increased the availability of muscle carnitine (hypercarnitinemia) in conjunction with hyperinsulinemia to determine if there would be a decline in fat oxidation rates during high CHO availability (as induced by hyperinsulinemia). It was found that with a 15% increase in skeletal muscle carnitine content there was a significant decrease in pyruvate dehydrogenase complex activity, and a 40% decrease in muscle lactate content. After an overnight fast there was also an increase in LCA-CoA and glycogen content. These findings indicate that increased muscle carnitine decreased glycolytic flux, and CHO oxidation, and resulted in glucose being stored (Stephens *et al.* 2006).

From these studies it seems that the amount of available carnitine is important in the regulation of the interaction of CHO and lipid metabolism; specifically, its increase or decrease is associated with a decrease or increase in CHO metabolism. Carnitine's

regulatory role or lack thereof with CPT-I activity has not been full elucidated and requires further research.

4.3.4 Role of FAT/CD36

Studies have shown that FAT/CD36 is involved in the regulation of FA metabolism. FAT/CD36 is present in the sarcolemma, intracellular pool, and mitochondria of muscle cells of rats and humans, and is involved in the transport and uptake, (Ibrahimi, 1999; Campbell *et al.*, 2004; Holloway *et al.*, 2006) and oxidation of LCFA's (Campbell *et al.*, 2004; Holloway *et al.*, 2006). Ibrahimi *et al.*, (1999) showed that the overexpression of FAT/CD36 in mice muscle tissue results in an enhanced ability to take up and oxidize LCFA's in the soleus muscle in response to stimulation-induced muscle contraction. Similarly, Campbell *et al.*, (2004) demonstrated using an inhibitor of FAT/CD36, that palmitate binding and oxidation was suppressed in rat mitochondria therefore identifying that FAT/CD36 is involved in the uptake and transport of LCFA's across the mitochondrial membrane. An increase in the rate of fatty acid oxidation by inducing 30 minutes of electrical stimulation also resulted in an increased amount of FAT/CD36 protein expression (Campbell, 2004).

In human skeletal muscle during 120 minutes of cycling at 60%VO_2max, mitochondrial FAT/CD36 protein increased and was found to be correlated with both palmitate oxidation rates and whole body oxidation rates (Holloway *et al.*, 2006). Also, the use of an FAT/CD36 inhibitor resulted in reduced mitochondrial palmitate oxidation rates (Holloway *et al.*, 2006). Clearly, FAT/CD36 plays an important role in LCFA

transport, uptake, and oxidation in the muscle cell during exercise however the mechanism by which this transporter accomplishes these tasks is unknown.

4.3.5 Catecholamine levels

Catecholamine (epinephrine and norepinephrine) levels are increased during exercise (Hartley *et al.*, 1972; Winder *et al.*, 1978). They are important in fat metabolism because they induce lypolysis by activating hormone sensitive lipase (HSL) through β_2-adrenergic stimulation. Also, during high intensity exercise, epinephrine has been shown to increase glycogenolysis in skeletal muscle and also cause hyperglycemia in rats (Marker *et al.*, 1986). In resting rat muscle, epinephrine has different effects in different muscle types. In more oxidative muscles (soleus), epinephrine was shown to stimulate triglyceride hydrolysis over glycogenolysis whereas in the glycolytic muscle (epitrochlearis), the reverse occurred (Peters *et al.*, 1998).

In humans, Hartley *et al.*, 1972 demonstrated in that bicycling at 73%VO_2max elicited significant increases in norepinephrine levels compared to rest after 40 minutes of exercise and at exhaustion; after endurance training, norepinephrine levels were attenuated compared to pretraining levels (Hartley *et al.*, 1972). Similarly, Winder *et al.*, 1978 demonstrated that both epinephrine and norepinephrine levels were significantly higher than at rest when subjects rode at 95 to 100% of their VO_2max for 5 minutes. After 7 weeks of endurance training, those levels decreased considerably when they exercised at the same pre-training work load.

Catecholamine levels would definitely have an impact on fuel utilization in the HCR's and LCR's, especially at 70 and 80%VO_2max. The HCR's greater endurance

capacity may attenuate catecholamine levels compared to the LCR's and as a result, the different hormonal effects may influence fuel utilization.

4.3.6 Interaction of lipids and CHO

Fatty acid oxidation occurred maximally at 60%VO_2max in both groups of rats and as exercise intensity increased, lipid oxidation decreased and CHO oxidation took over as the dominant fuel (Fig. 4A,B, 6C,D). The mechanism behind this change in fuel use is not well known as mentioned above however substrate (lipid and CHO) availability and oxidation have been shown to affect overall fuel use. The Randle cycle postulates that increased fat oxidation decreases glycolytic flux by inhibiting PDH and PFK activities, through an increase in the acetyl-CoA/CoA ratio and citrate concentration respectively. Other studies have shown that increasing either substrate (fat or CHO) inhibits the use of the other substrate as fuel (see Introduction 1.3.3). In the present study, it is possible that at the point of maximal lipid oxidation (60%VO_2max), glycolytic flux decreases through the inhibition of PDH and PFK activities (Randle cycle). As exercise intensity increases past 60%VO_2max, glycolytic flux increases, possibly by an increase in malonyl-CoA concentration or PDHa activity due to a reduced NADH/NAD^+ ratio or increase in Ca^{2+}.

4.4 SKELETAL MUSCLE ADAPTATIONS

4.4.1 Increased oxidative capacity in the HCR's

The HCR's have a significantly higher aerobic (VO_2max) and endurance capacity (distance run to exhaustion) than the LCR's (Table 1). The HCR's also oxidize a greater amount of lipids and CHO than the LCR's at all the exercise intensities tested but not all

the differences were significant (see Fig.4). Since the HCR's have a greater endurance capacity than the LCR's it is expected that they would have adaptations in their skeletal muscle that would allow them to oxidize greater amounts of fuel. Measuring enzymes indicative of mitochondrial content and oxidative capacity revealed that there were higher CS,COX, and HOAD enzyme activities in the HCR's compared to the LCR's in the soleus (CS and COX) and gastrocnemius (HOAD; Table 3). These results are somewhat similar to those found in the seventh generation of the same line of rats and in endurance-trained humans (see Introduction 1.4.2; Howlett *et al.*, 2002).

In the HCR's and LCR's of the seventh generation, Howlett *et al.* (2002) investigated the factors responsible for improved running endurance and increased oxygen utilization in the HCR's. VO_2max and muscle O_2 conductance were found to be higher in the HCR's versus the LCR's and the adaptations for improved endurance running capacity were found mainly in the skeletal muscle (gastrocnemius). Mitochondrial enzyme activity (CS and HOAD) was significantly higher and PFK activity was significantly lower in the HCR's compared to the LCR's at rest (Howlett *et al.*, 2002). This study supports the findings of Howlett *et al.*, (2002) because both CS and HOAD activities were higher in the HCR's compared to the LCR's thereby showing that the HCR's do have a higher oxidative capacity.

The HCR's have some skeletal muscle adaptations for a higher oxidative capacity than the LCR's as seen from the increased activity of oxidative enzymes in both the soleus and gastrocnemius, accounting for the greater distance to exhaustion (Table 1). For VO_2max, higher values have been shown to be achieved by both central (O_2 delivery) and peripheral (skeletal muscle) adaptations but O_2 delivery is the main limiting factor

(Bassett and Howley, 1999). In the seventh generation of this rat model, VO_2max increased due to greater extraction (capillary to muscle cell) and utilization of O_2 by the muscle (mostly peripheral adaptations) because of a higher capillary density and increased oxidative enzymes (Gonzalez *et al.*, 2005). As a result, in this study, it is expected that along with skeletal muscle adaptations, the HCR's would have a greater capillary density than the LCR's. This would aid in the delivery of circulatory fuel sources (plasma fatty acids and glucose).

4.4.2 Fewer metabolic disturbances during exercise in the HCR's

Measurements of ATP and PCr, concentrations revealed that exercise at 60%VO_2max for one hour did not result in a significant change in energy charge (aside from the EDL) during exercise within and between the LCR's and HCR's (Table 4). One explanation of this may be that the intensity of the exercise (60%VO_2max) may not have been high enough to elicit any changes in metabolism after one hour. If the HCR's and LCR's were to run at an intensity of 80%VO_2max or higher, there would probably be differences in ATP and PCr concentrations between the groups; the LCR's would deplete more PCr and ATP than the HCR's due to their decreased oxidative capacity.

Endurance trained mammals 's have a higher oxidative capacity than their untrained counterparts; those studies will be used to support the notion that the HCR's have a higher oxidative capacity and therefore may have attenuated ATP and PCr levels compared to the LCR's. It has been shown in both humans and rats that endurance training results in fewer fluctuations in energy metabolism compared to pre-training (Holloszy and Coyle, 1984; Holloszy and Booth, 1976; Constable *et al.*, 1987). This is

mainly due to an increased amount of mitochondria in trained muscle. More mitochondria means that there are a greater number of respiratory chains to consume the same amount of O_2 at the same work rate in the trained muscle versus the untrained muscle; therefore, each respiratory chain in the trained muscle would not have to do as much work (ie. less O_2 would be consumed) to achieve the same rate of O_2 consumption. Similarly, training increases enzyme levels per gram of muscle and this would decrease the rate of substrate flux because there are more enzymes to handle the energy demand. As a result, the concentration of the regulators of the enzymes in the pathway (activators, cofactors, etc.) would not fluctuate as much (Holloszy and Coyle, 1984). A steady work rate that is achieved by a trained muscle would allow for decreased metabolic fluctuations compared to untrained muscle (Holloszy and Booth, 1976).

In support of this, sedentary and endurance-trained rats (12 wks, 2 hrs/day, 5days/wk) underwent muscle stimulation for 8 minutes. It was found that compared to sedentary rats, the trained rats had smaller decreases in PCr and ATP concentrations and stabilized at 3 minutes with no further decreases in concentration during the remaining 5 minutes of stimulation (Constable et al., 1987). In humans, Karlsson *et al.*, 1972 demonstrated that training (running 5-6 km, 2-3 x/wk) increased resting values of ATP and the depletion of ATP was reduced after training compared to pre-training.

At a higher relative exercise intensity, it is expected that the HCR's would have fewer disturbances in metabolism therefore concentrations of ATP and PCr would not decrease as much as in the LCR's because of their higher oxidative capacity.

4.4.3 Fiber recruitment and exercise intensity

Muscle fibers seem to be recruited in a coordinated fashion with increasing exercise intensity. The size of the muscle's alpha-motorneurons (located within the motor unit) determines the recruitment order of muscle fibers (Armstrong, 1988). Typically, motorneurons are recruited from small to large, innervating slow and then fast twitch fibers respectively (Armstrong, 1988). The function of the motorneurons is matched by the metabolic properties of the muscle fiber which are described in the introduction (1.4.5). Briefly, slow twitch red (type I) fibers have a high oxidative capacity (and greater ability to oxidize fat) and therefore are used primarily during low intensity exercise when energy is produced aerobically (Mrad *et al.*, 1992; Sahlin *et al.*, 2006). Fast twitch white (type II) fibers are recruited to power exercise at higher exercise intensities when CHO utilization dominates (Armstrong and Laughlin, 1985). At exercise intensities above maximum oxygen uptake, both slow and fast twitch fibers are recruited; however the fast twitch fibers comprise the greatest proportion of the recruited mass (Gollnick *et al.*, 1974).

Muscle fibers are also very adaptable since training has been shown to have an effect on fiber composition and recruitment. Endurance-trained humans have more type I fibers than untrained individuals (Gollnick, 1972). Also, the oxidative capacity of both main fiber types (type I and II) increases with endurance training (Gollnick, 1972). Type II fibers, that were once subject to rapid fatigue and lactate accumulation during high exercise intensities because of a low aerobic capacity, are able to maintain contraction for a longer period of time under the same pre-training conditions (Holloszy and Coyle, 1984).

Muscle fibers are recruited according to the energy demand being placed on the muscle and this is also true with CHO and lipid utilization. Since the HCR's have a greater capacity for oxidation than the LCR's it is reasonable to predict that they may have a greater amount of type I fibers. This however, may not be the case since in the 15^{th} generation of these rats, no differences in fiber type were found between the two groups in the soleus muscle (Walsh et al., 2006).

4.5 GENETIC AND ENVIRONMENTAL CONSIDERATIONS

This study shows that the LCR's have a lower endurance capacity than the HCR's. Wisloff *et al.* (2005), has shown that the 7^{th} generation of these rats have a greater chance of developing CVD due to many metabolic abnormalities including increased mean blood pressure, higher fasting blood glucose, and insulin resistance (see 1.5.2 introduction); there is a possibility that the LCR's in this study may have those metabolic abnormalities. These traits coupled with physical inactivity and alterations in protein expression would put the LCR's at risk for CVD (Booth and Lees, 2006). The HCR's, who have demonstrated a high endurance capacity and corresponding skeletal muscle adaptations without any training, provide evidence that evolution has selected metabolic pathways that are associated with physical activity because they confer increased fitness during the course of time (Booth and Lees, 2006). This model helps us to understand which physiological traits are important for increased endurance capacity and possibly which traits lead to the development of disease.

4.6 CONCLUSIONS

Rats selected for their intrinsic endurance running capacity (high and low) do follow the pattern of fuel selection seen in other mammals tested so far; they oxidize the same proportions of CHO and lipids when exercising at the same percentage of their VO_2max. Also, CHO oxidation rates increase with increasing exercise intensity while lipid oxidation rates decrease concomitantly. The HCR's have a higher oxidative capacity as seen by their higher VO_2max, greater distance to exhaustion, greater total rates of oxidation, and higher activities of CS, COX, and HOAD. In other words, rats that are bred to have a higher endurance capacity have adaptations at the skeletal level that allows them to oxidize a greater amount of CHO and lipids. The LCR's represent a potential model of disease due to their inactivity combined with physiological factors associated with disease, while the HCR's provide insight into the physiological characteristics that have persisted through generations of selection and therefore confer greater fitness.

4.7 FUTURE DIRECTIONS

This study used a unique rat genetic model to examine the effects of exercise intensity on lipid and CHO utilization. Futhermore, it began an investigation into the mechanisms involved (at the level of the skeletal muscle) in understanding the relationship between CHO and lipid metabolism, maximal fat oxidation, and overall fuel selection patterns that are determined by the intensity of the exercise performed. Current research suggests that specific enzymes and transporters, and fiber types play

a major role in fuel selection and should be investigated further. Further research could definitely be accomplished with these rats to provide further insight into the mechanisms. For example, pyruvate dehydrogenase activity (indicative of glycolytic flux), CPT-I, malonyl-CoA, carnitine, glycogen (fiber recruitment), and blood lactate concentrations are just a few metabolic factors that could be measured.

REFERENCES

Abou Mrad, J., Yakubu, F., Lin, D., Peters, J.C., Atkinson, J.B., and J.O. Hill. 1992. Skeletal muscle composition in dietary obesity-susceptible and dietary obesity-resistant rats. Am J Physiol Regulatory Integrative Comp Physiol 262: 684-688.

Achten, J., Gleeson, M., and A.E. Jeukendrup. 2002. Determination of the exercise intensity that elicits maximal fat oxidation. Med Sci Sports Exercise 34(1): 92-7.

Achten, J., and A.E. Jeukendrup. 2003. Maximal Fat Oxidation During Exercise in Trained Men. Int J Sports Med 24: 603-608.

Ahlborg, G., Felig, P., Hagen feldt, L., Hendler, R., and J. Wahren. 1974. Substrate turnover during prolonged exercise in man. Splanchnic and leg metabolism of glucose, free fatty acids, and amino acids. J Clin Invest 53(4): 1080-90.

Armstrong, R.B. 1988. Muscle Fiber Recruitment Patterns and their Metabolic Correlates In: Exercise, Nutrition, and Energy Metabolism ed. Horton E.S., and Terjung, R.L. MacMillan Pub.Co.NY.

Armstrong, R.B., and M.H. Laughlin. 1985. Metabolic Indicators of Fibre Recruitment in Mammalian Muscles During Locomotion. J. exp. Biol. 115: 201-213.

Baar, K., Wende, A.R., Jones, T.E., Marison, M., Nolte, L.A., Chen, M., Kelley, D.P., and J.O. Holloszy. 2002. Adaptations of skeletal muscle to exercise: rapid increase in the transcriptional co-activator PGC-1. FASEB J 16(4): 1879-86.

Bassett, D.R. Jr., and E.T. Howley. 2000. Limiting factors for maximum oxygen uptake and determinants of endurance performance. Med Sci Sports Exerc. 32(1): 70-84.

Bergman, B.C., Butterfield, G.E., Wolfel, E.E, Lopaschuk, G.D., Casazza, G.A., Horning, M.A., and G.A. Brooks. 1999. Muscle net glucoe uptake and glucose kinetics after endurance training in men. Am J Physiol 277 Endocrinol Metab 40: E81-E92.

Bezaire, V., Heigenhauser, G.J.F., and L.L. Spriet. 2003. Regulation of CPT I activity in intermyofibrillar and subsarcolemmal mitochondria from human and rat skeletal muscle. Am J Physiol Endocrinol Metab 286: E85-E91.

Blaak, E.E. 2004. Basic disturbances in skeletal muscle fatty acid metabolism in obesity and type 2 diabetes meillitus. Proceedings of the Nutrition Society 63: 323-330.

Bloomstrand, E., Ekblom, B., and E.A. Newsholme. 1986. Maximum activities of key glycolytic and oxidative enzymes in human muscle from differently trained individuals. J Physiol 381: 111-118.

Booth, F.W. and S.J. Lees. 2007. Fundamental questions about genes, inactivity, and chronic diseases. Physiol Genomics 28: 146-157.

Bouchard, C., An, P., Rice, T., Skinner, J.S., Wilmore, J.H., Gagnon, J., Perusse, L., Leon, A.S., and D.C. Rao. 1999. Familial aggregation of VO2max response to exercise training: results from the HERITAGE Family Study. J Appl. Physiol. 87(3): 1003-1008.

Brooks, G.A. and J. Mercier. 1994. Balance of carbohydrate and lipid utilization during exercise: the "crossover" concept. J. Appl. Physiol. 76(6): 2253-2261.

Campbell, S.E., Tandon, N.N., Woldeiorgis, G., Luiken, J.J.F.P., Glatz, J.F.C., and A. Bonen. 2004. A novel function for fatty acid translocase (FAT)/CD36. The Journal of Biological Chemistry 279(35): 36235-36241.

Carter, S.L., Rennie, C.D., Hamilton, S.J., and M.A. Tarnopolsky. 2001. Changes in skeletal muscle in males and females following endurance training. Can.J.Physiol.Pharmacol.29: 386-392.

Chien, D., Dean, D., Saha, A.K., Flatt, J.P., and N.B. Ruderman. 2000. Malonyl –CoA content and fatty acid oxidation in rat muscle and liver in vivo. Am J Physiol Endocrinol Metab 279: E259-E265.

Chin, E.R. 2005. Role of Ca2+/calmodulin-dependent kinases in skeletal muscle plasticity. J Appl Physiol 99: 414-23.

Constable, S.H., Favier, R.J., McLane, J.A., Fell, R.D., Chen, M., and J.O. Holloszy. 1987. Energy metabolism in contracting rat muscle: adaptation to exercise training. Am J Physiol Cell Physiol 22: C316-C322.

Coyle, E.F., Coggan, A.R., Hopper, M.K., and T.J. Walters. 1988. Determinants of endurance in well-trained cyclists. J Appl Physiol 64(6): 2622-30.

Coyle, E.F., Jeukendrup, A.E., Wagenmakers, A.J., and W.H. Saris. 1997. Fatty acid oxidation is directly regulated by carbohydrate metabolism during exercise. Am J Physiol Endocrinol Metab 273(36): E268-75.

Delp, M.D., and C. Duan. 1996. Composition and size of type I, IIA, IID/X, and IIB fibers and citrate synthase activity of rat muscle. J Appl Physiol 80(1): 261-270.

Dohm, G.L., Williams, R.T., Kasperek, G.J., and A.M. vanRij. 1982. Increased excretion of urea and N-methylhistidine by rats and humans after a bout of exercise. J Appl Physiol: Respirat Environ Exercise Physiol 52(1): 27-33.

Donovan, C.M. and G.A. Brooks. 1983. Endurance training affects lactate clearance, not lactate production. J Physiol Endocrinol Metab 244(7): E83-E92.

Donovan C.M. and M.J. Pagliassotti. 1990. Enhanced efficiency of lactate removal after endurance training. J Appl Physiol 68(3): 1053-58.

Febbraio, M.A. and J. Dancey. 1999. Skeletal muscle energy metabolism during prolonged fatiguing exercise. J Appl Physiol 87(6): 2341-7.

Felic, P., and J. Wahren. 1975. Fuel Homeostasis in Exercise. The New England Journal of Medicine. 293(21): 1078-1084.

Ferrannini, E. and A. Mari. 1998. How to measure insulin sensitivity. Journal of Hypertension. 16: 895-905.

Frayn, K.N. 1983. Calculation of substrate oxidation rates in vivo from gaseous exchange. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 55(2): 628-634.

Friedlander, A.L., Casazza, G.A., Horning, M.A., Buddinger, T.F., and G.A. Brooks. 1998. Effects of exercise intensity and training on lipid metabolism in young women. Am J Physiol Endocrinol Metab 275: E853-E863.

Gibala, M.J. 2001. Regulation of Skeletal Muscle Amino Acid Metabolism During Exercise. International Journal of Sport Nutrition and Exercise Metabolism 11:87-108.

Gollick, P.D., Armstrong, R.B., Saubert, C.W., Piehl, K, and B.Saltin. 1972. Enzyme Activity and fiber composition in skeletal muscle of untrained and trained men. Journal of Applied Physiology 33(3): 312-319.

Gonzalez, N.C., Howlett, R.A., Henderson, K.K., Koch, L.G, Britton, S.L., Wagner, H.E., Fayret, F., and P.D. Wagner. 2006. Systemic oxygen transport in rats artificially selected for running endurance. Respir Physiol Neurobiol 151(2-3): 141-50.

Hartley, L.H., Mason, J.W., Hogan, R.P, Jones, L.G., Kotchen, T.A., Mougey, E.H., Wherry, F.E., Pennington, L.L., and P.T. Ricketts. 1972. Multiple hormone responses to graded exercise in relation to physical training. J Appl Physiol 33(5): 602-6.

Hayes, J.P. and C.S. O'Connor. 1999. Natural Selection on Thermogenic Capacity of High-Altitude Deer Mice. Evolution 53(4): 1280-1287.

Henderson, S.A., Black, A.L. and G.A. Brooks. 1985. Leucine Turnover and oxidation in trained rats during exercise. Am J Physiol Endocrinol Metab 249(12): E137-E144.

Holloszy, J.O., and F.W. Booth. 1976. Biochemical adaptations to endurance exercise in muscle. Annu Rev Physiol 38: 273-91.

Helge, J.W., Watt, P.W., Pichter, E.A., Rennie, M.J., and B. Kiens. 2001. Fat utilization during exercise: adaptation to a fat-rich diet increases utilization of plasma fatty acids and very low density lipoprotein-triacylglycerol in humans. J Physiol 537(3): 1009-1020.

Henriksson, J. 1976. Training induced adaptation of skeletal muscle and metabolism during submaximal exercise. J Physiol 270: 661-675.

Holloszy, J.O. and E.F. Coyle. 1984. Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. J. Appl. Physiol: Resprat. Environ. Exercise Physiol. 56(4): 831-836.

Holloszy, J.O., Kohrt, W.M., and P.A. Hansen. 1998. The regulation of carbohydrate and fat metabolism during and after exercise. Frontiers in Bioscience 3: d1011-1027.

Holloway, G.P., Bezaire, V., Heigenhauser, G.J.F., Tandon, N.N, Glatz, J.F.C., Luiken, J.J.F.P., Bonen, A., and L.L. Spriet. 2006. Mitochondrial long chain fatty acid oxidation, fatty acid translocase/CD36 content, and carnitine palmitoytransferase I activity in human skeletal muscle during aerobic exercise. J Physiol 571(1): 201-210.

Houle-leroy, P., Garland, T, Jr., Swallow, J.G., and H. Guderley. 2000. Effects of voluntary activity and genetic selection on muscle metabolic capacities in house mice *Mus domesticus*. J Appl Physiol 89: 1608-1616.

Howlett, R.A., Gonzalez, N.C., Wagner, H.E., Fu, W., Britton, S.L., Koch., L.G., and Wagner, P.D. 2002. Genetic Models in Applied Physiology: Selected Contribution: Skeletal muscle capillarity and enzyme activity in rats selectively bred for running endurance. J Appl Physiol 94: 1682-1688.

Hultman, E. and L.L. Spriet. 1988. Dietary intake prior to and during exercise. In Exercise, Nutrition, and Energy Metabolism (Horton, E.S. and Terjung, R.L., eds.), pp. 132-149, Macmillan, New York.

Ibrahimi, A., Bonen, A., Bliner, W.D., Hagri, T., Li, X., Zhong, K., Cameron, R., and N.A. Abumrad. 1999. Muscle-specific Overexpression of FAT/CD36 Enhances Fatty Acid Oxidation by Contracting Muscle Reduces Plasma triglycerides and Fatty Acids, and Increases Plasma Glucose and Insulin. The Journal of Biological Chemistry 274(38): 26761-26766.

Jeukendrup, A.E. 2002. Regulation of fat metabolism in skeletal muscle. Ann. N.Y. Acad. Sci. 967: 217-235.

Jeukendrup, A.E., and G.A. Wallis. 2005. Measurement of Substrate Oxidation During Exercise By Means of Gas Exchange Measurements. Int J Sports Med 26(Suppl 1): 528-537.

Kelley DE & Simoneau JA (1994) Impaired free fatty acid utilization by skeletal muscle in non-insulin-dependent diabetes mellitus. Journal of Clinical Investigation 94: 2349–2356.

Kerner, J., and C. Hoppel. 2000. Fatty acid import into mitochondria. Biochimica et Biophysica Acta 1486(1): 1-17.

Kiens, B., 2006. Skeletal muscle lipid metabolism in exercise and insulin resistance. Physiol Rev 86:205-243.

Kim, J.Y., Koves, T.R., Yu, G-S., Gulick, T., Cortright, R.N., Dohm, G.L., and D.M, Muoio. 2001. Evidence of a malonyl-CoA-insensitive carnitine palmitoyltransferase I activity in red skeletal muscle. Am J Physiol Endocrinol Metab 282: E1014-E1022.

Klissouras, V. 1971. Heritability of adaptive variation. J Appl Physiol 31(3): 338-44.

Koch, L.G. and S.L.Britton. 2001. Artificial selection for intrinsic aerobic endurance running capacity in rats. Physiol Genomics 5: 45-52.

Koubi, H.E., Desplanches, D., Gabrielle, C., Cottet-Emard, J.M., Sempore, B., Favier, R.J. 1991. Exercise endurance and fuel utilization: a reevaluation of the effects of fasting. 70(3): 1337-43.

LeBlanc, P.J., Howarth, K.R., Gibala, M.J., and G.J.F. Heigenhauser. 2004. Effects of 7wk of endurance training on human skeletal muscle metabolism during submaximal exercise. J Appl Physiol 97:2148-53.

Luiken, J.J.F.P., Bonen, A., and J.F.C. Glatz. 2002. Cellular fatty acid uptake is acutely regulated by membrane-associated fatty acid-binding proteins. Prostaglandins, Leukotrienes and Essential Fatty Acids 67(2-3): 73-78.

Karlsson, J., Nordesjo, L-O., Jorfeldt, L., and B.Saltin. 1972. Muscle lactate ATP, and CP levels during exercise after physical training in man. J Appl Physiol 33(2): 199-203.

Kraegen, E.W., Saha, A.K., Preston, E., Wilks, D., Hoy, A.J., Cooney, G.J., and N.B. Ruderman. 2006. Increased malonyl-CoA and diacylglycerol content and reduced AMPK activity accompany insulin resistance induced by glucose infusion in muscle and liver in rats. Am J Physiol Endocrionol Metab 290:471-479.

Krssak, M., Petersen, K.F., Bergeron, R., Price, T., Laurent, D., Rothman, D.L., Roden, M., and G.I. Shulman. 2000. Intramuscular glycogen and intramyocellular lipid utilization during prolonged exercise and recovery in man: a 13C and 1H nuclear magnetic resonance spectroscopy study. J Clin Endocrinol Metab 85(2): 748-54.

MacRae, H.S.H., Dennis, S.C., Bosch, A.N., and T.D. Noakes. 1992. Effects of training on lactate production and removal during progressive exercise in humans. J Appl Physiol 72(5): 1649-56.

McClelland, G. 2004. Fat to the fire: the regulation of lipid oxidation with exercise and environmental stress. Comparative Biochemistry and Physiology, Part B 139: 443-460.

McClelland, G., Hochachka, P.W. and J.M. Weber. 1998. Carbohydrate utilization during exercise after high-altitude acclimation: A new perspective. Proc. Natl. Acad. Sci. 95: 10288-10293.

McClelland, G., Hochachka, P.W. and J.M. Weber. 1999. Effect of high-altitude acclimation on NEFA turnover and lipid utilization during exercise in rats. Am J Physiol Endocrinol Metab 277 (40): E1095-E1102.

McClelland, G., Zwingelstein, G., Taylor, C.R., and J-M. Weber. 1994. Increased capacity for circulatory fatty acid transport in a highly aerobic mammal. Am J Physiol Regulatory Integrative Comp Physiol 266(35): R1280-R1286.

McKenzie, S., Phillips, S.M., Carter, S.L., Lowther, S., Gibala, M.J., and M.A. Tarnopolsky. 2000. Endurance exercise training attenuates leucine oxidation and BCOAD activation during exercise in humans. Am J Physiol Endocrinol Metab 278: E580-E587.

Nelson, D.L. and M.M. Cox. Lehninger Principles of Biochemistry 3rd ed. Worth Publishers: New York, 2000.

Odland, L.M., Heigenhauser, G.J.F., Lopaschuk, G.D., and L.L. Spriet. 1996. Human skeletal muscle malonyl-CoA at rest and during prolonged submaximal exercise. Am J Physiol Endocrinol Metab 270(33): E541-E544.

Odland, L.M., Heigenhauser, G.J., Wong, D., Hollidge-Horvat, M.G., and L.L. Spriet. 1998. Effects of increased fat availability on fat-carbohydrate interaction during prolonged exercise in men. Am J Physiol Regul Integr Comp Physiol 274: R894-R902.

Ojuka, E.O., Jones, T.E., Han, D.H., Chen, M., Wamhoff, B.R., Sturek, M., and J.O. Holloszy. 2002. Intermittent increases in cytosolic Ca2+ stimulate mitochondrial biogenesis in muscle cells. Am J Physiol Endocrinol Metab 283(5): E1040-5.

Peinado, B, Latorre R, Va´quez-Auto´n, J.M., , Poto, A, Rami´rez, G, Lo´pez-Albors, O, Moreno, F. and F. Gil. 2003. Histochemical Skeletal muscle Fibres in the Sheep. Anat. Histol. Embryol. 33: 236–243.

Peters, S.J., Dyck, D.J, Bonen, A., and L.L Spriet. 1998. Effects of epinephrine on lipid metabolism in resting skeletal muscle. Am J Physiol Endocrinol Metab 275(38): E300-E309.

Philips, S.M., Atkinson, S.A., Tarnopolsky, M.A., and J.D. MacDougall. 1993. Gender differences in leucine kinetics and nitrogen balance in endurance athletes. J Appl Physiol 75: 2134-2141.

Powers, S.K. and E.T. Howley. Exercise Physiology 3rd ed. McGraw-Hill: 1996.

Randle, P.J., Garland, P.B, Hales, C.N., Newsholme, E.A., Denton, R.M, and C.I. Pogson. 1963. The glucose fatty acid cycle: it's role in insulin sensitivity and the metabolic disturbances of diabetes meillitus. Lancet i: 785-789.

Rennie, M.J., Bohe, J., Smith, K., Wackerhage, H., and P. Greenhaff. 2006. Branched-Chain Amino Acids: Metabolism, Physiological Function, and Application. J Nutr 136: 264S-268S.

Roberts, T.J., Weber, J-M., Hoppeler, H., Weibel, E.R., and C.R. Taylor. 1996. Design of the oxygen and substrate pathways II. Defining the upper limits of carbohydrate and fat oxidation. The Journal of Experimental Biology 199, 1651-1658.

Romijn, J.A, Coyle, E.F., Hibbert, J., and R.R. Wolfe. 1992. Comparison of indirect calorimetry abd a new breath 13C/12C ratio method during strenuous exercise. Am J Physiol 263 Endocrinol Metab 26: E64-E71.

Romijn, J.A., Coyle, E.F., Sidossis, L.S., Gastaldelli, A., Horowitz, J.F., Endert, E., and R.R. Wolfe. 1993. Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. Am. J. Physiol. 265 (Endocrinol. Metab. 28): E380-E391.

Romijn, J.A., Coyle, E.F., Sidossis, L.S., Zhang, X-J., and R.R. Wolfe. 1995. Relationship between fatty acid delivery and fatty acid oxidation durin strenuous exercise. Appl Physiol 79(6): 1939-1945.

Rose, A.J., and E.A. Richter. 2005. Skeletal Muscle Glucose Uptake During Exercise: How is it Regulated? Physiology 20:269-270.

Saha, A.K., Vavvas, D., Kurowski, T.G., Apazidis, A., Witters, L.A., Shafrir, E., and N.B. Ruderman. 1997. Malonyl-CoA regulation in skeletal muscle: its link to cell citrate and the glucose fatty acid cycle. Am J Physiol Endocrinol Metab 272(35): E641-48.

Sahlin, K., Mogenson, M., Bagger, M., Fernstrom, M., and P.K. Pederson. 2006. the potential for mitochondrial fat oxidation in human skeletal muscle influences whole body fat oxidation during low inetsnity exercise. Am J Physiol Endocrinol Metab 292: 223-230.

Sahlin, K., Soderland, K., Tonkonogi, M., and K. Hirakoba. 1997. Phosphocreatine content in single fibers of human muscle after sustained submaximal exercise. Am. J. Physiol. 273 (Cell Physiol. 42): C172-C178.

Sorrentino, M.J. 2005. Implications of the metabolic syndrome: the new epidemic. Am J Cardiol 96(4A): 3E-7E.

Spriet LL, and Howlett RA. Metabolic control of energy production during physical activity. In: Lamb DR and R Murray (eds.). Perspectives in Exercise Science and Sports

Medicine, Vol. 12: The Metabolic Basis of Performance in Exercise and Sport. Carmel, IN: Cooper, 1999, pp. 1-51.

Spriet, L.L., Howlett, R.A., and G.J.F. Heigenhauser. 2000. An enzymatic approach to lactate production in human skeletal muscle during exercise. Med. Sci. Sports Exerc. 32(4): 756-763.

Stellingwerff, T., Watt, M.J., Heigenhauser, G.J., and L.L. Spriet. 2003. Effects of reduced free fatty acid availability on skeletal muscle PDH activation during aerobic exercise. Pyruvate dehydrogenase. Am J Physiol Endocrinol Metab 284(3): E589-96.

Stevens, F.B., Constantin-Teodosiu, D., Laithwaite, D., Simpson, E.J., and P.L. Greenhaff. 2006. An acute increase in skeletal muscle carnitine content alters fuel metabolism in resting human skeletal muscle. The Journal of Clinical Endocrinology and Metabolism 91(12): 5013-5018.

Stephens, F.B., Constantin-Teodosiu, D., and P.L. Greenhaff. 2007. New insight concerning the role of carnitine in the regulation of fuel metabolism in skeletal muscle. J Physiol In Press.

Stich, V. and M. Berlan. 2004. Physiological Regulation of NEFA Availability: Lipolysis Pathway. Proceedings of the Nutrition Society 63: 361-374.

Stisen, A.B, Stougard, O, Langfort, J., Helge, J.W., Sahlin, K., and K. Madsen. 2006. Maximal fat oxidation rates in endurance trained and untrained women. Eur J Appl Physiol 98(5): 497-506.

van Loon, L.J.C., Greenhaff, P.L., Constantin-Teodosiu, D., Saris, W.H.M. and A.J.M. Wagenmakers. 2001. The effects of increasing exercise intensities on muscle fuel utilization in humans. J Physiol 536: 295-304.

van Loon, L.J.C, Koopman, R., Stegen, J.H.C.H., Wagenmakers, A.J.M., Keizer, H.A., and W.H.M. Saris. 2003. Intramyocellular lipids form an important substrate during moderate intensity exercise in endurance-trained males in a fasted state. J Physiol 553: 611-625.

Walsh, B., Hooks, R.B., Hornyak, J.E., Koch, L.G., Britton, S.L. and M.C. Hoggan. 2006. Enhanced mitochondrial sensitivity to creatine in rats bred for high aerobic capacity. J Appl Physiol 100(6): 1765-9.

Wang, Y.X., Zhang, C.L., Yu, R.T., Cho, H.K., Nelson, M.C., Bayuga-Ocampo, C.R., Ham, J., Kang, H., and R.M. Evans. 2005. Regulation of muscle fiber type and running endurance by PPARdelta. PLoS Biol 2(10): e294.

Weber, J-M., Brichon, G., Zwingelstein, G., McClelland, G., Saucedo, C., Weibel, E.R., and C.R. Taylor. 1996. Design of the oxygen and substrate pathways IV. Partitioning

Energy Provision From Fatty Acids. The Journal of Experimental Biology 199, 1667-1674.

Winder, W.W., Hagberg, J.M., Hickson, R.C., Ehsani, A.A., and J.A. McLane. 1978. Time course of sympathoadrenal adaptation to endurance exercise training in man. J Appl Physiol 45(3): 370-4.

Winder, W.W., Arogyasami, J., Barton, R.J., Elayan, I.M. and R.R. Vehrs. 1989 Muscle malonyl-CoA decreases during exercise. J Appl Physiol 67(6): 2230-3.

Wisloff, U., Najjar, S.M., Ellingsen, O., Haram, P.M., Swoap, S., Al-Share, Q., Fernstrom. M., Rezaei, K., Lee, S.J., Koch, L.G., and S.L. Britton. 2005. Cardiovascular Risk Factors Emerge After Artificial Selection for Low Aerobic Capacity. Science. 307: 418-420.

Withers, P.C. 1977. Measurement of VO2, VCO2, and evaporative water loss with a flow-through mask. J.Appl.Physiol.: Respirat. Environ. Exercise Physiol. 42(1): 120-123.

Wolfe, R.R., Klein, S., Carraro, F., and J-M. Weber. 1990. Role of triglyceride-fatty acid cycle in controlling fat metabolism in humans during and after exercise. Am.J.Physiol. 258 (Endocrinol. Metab. 21): E382-E389.